APIOS TERRESTRIAL EFFECTS WORKGROUP REPORT:

RESULTS OF FIVE STUDIES TO DETERMINE THE POTENTIAL EFFECTS OF SIMULATED ACID RAIN ON THE GROWTH OF SUGAR MAPLE AND WHITE SPRUCE SEEDLINGS

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Summary of Results

Simulated acidic rain (SAR) with pH 3.2, 4.3 and 5.6 was applied to wild sugar maple seedling transplants, as well as nursery-stock white spruce seedings, for 2.5 growing seasons. The multi-year study was carried out using a mobile rain exclusion canopy system located at the Phytotoxicology Controlled Environment Laboratory, Brampton, Ontario. No significant treatment effects were observed on growth parameters such as; seedling height, stem diameter, shoot dry weight, total leaf area, leaf pigments or foliar nutrient concentrations. Foliar sulphur and nitrogen concentrations were significantly higher in pH 3.2 SAR-treated seedlings due to higher inputs of these elements in pH 3.2 SAR. Overall, results did not show SAR had any detrimental effects on the growth or physiology of the tree seedling growth in this controlled field study.

An experiment was conducted concurrently with the above study to determine the effects of simulated acidic rain on the establishment of sugar maple seedlings. Sugar maple seedlings grown from stratified seeds in pots of mineral soil were exposed to SAR (pH 3.2, 4.3, 5.6) in the mobile rain exclusion canopy system. The establishment and early growth of the seedlings were not adversely affected by SAR applied over two growing seasons. In addition, a pilot experiment was designed to assess the suitability of root window chambers for investigating effects of soil acidification on sugar maple seedling root growth at the time of accelerated spring growth. SAR (pH 3.2, 4.3, 5.6) did not affect root development. It should be noted that spring root growth results from stored carbohydrates from previous years growth rather than from photosynthates produced during treatment application.

A ten week indoor greenhouse-chamber study was also conducted at the Phytotoxicology Controlled Environment Laboratory to investigate potential interaction effects of simulated acidic rain (SAR) and ozone fumigations on the growth and nutrient status of greenhouse-grown, two year old, white spruce seedlings. Experimental results indicated that growth of white spruce seedlings was not affected significantly by exposure to SAR, alone or in combination with ozone during the ten week study period, at the treatment concentrations applied.

EFFECTS OF SIMULATED ACID RAIN ON THE GROWTH, NUTRITION AND PHYSIOLOGY OF WHITE SPRUCE SEEDLINGS, GROWN IN THREE SOILS WITHIN A MOBILE RAIN EXCLUSION CANOPY SYSTEM

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ABSTRACT

White spruce (Picea glauca (Moench) Voss) seedlings were exposed to simulated acid rain of pH 3.2, 4.3 and 5.6 for two and one half growing seasons. The spruce seedlings were grown in three soils ranging from pH 4.8 to 6.0. Ambient rain was excluded from the treatment plots by mobile rain exclusion canopies.

A significant increase in the foliar sulphur concentration was observed in seedlings treated with pH 3.2 simulated acid rain (SAR). However, there were no significant changes to any other parameters measured to determine response to the treatments applied; ie. seedling growth, leaf area, leaf number, foliar pigment, nutrient concentrations or photosynthetic rate. No seedling mortality occurred after an initial establishment period. Needle chlorosis and necrosis was not observed at any treatment level. No measurable alterations in soil pH were recorded over the course of the study.

INTRODUCTION

Acidic rainfall has the potential to damage forest trees and has been implicated as a possible contributor to hardwood and conifer declines observed in Europe and North America (Ulrich et al., 1980; Ulrich, 1983; Lefohn and Brocksen, 1984; Hornbeck and Smith, 1985; Van Breemen, 1985). The actual effect of acid deposition on forest ecosystems is still unclear due to the complexity and diversity of these ecosystems. Research has increasingly focused on changes in soil chemistry and effects on root growth. Ulrich (1983) suggested that soil acidification from acidic deposition causes a loss of essential plant nutrients from soils, as well as mobilization of toxic ions like Al. Zottl and Huettl (1986) postulated that the decline of Norway spruce (Picea abies L.) in Germany is caused by ozone which results in physiological and anatomical damage to spruce foliage. This foliage is then susceptible to foliar leaching by acid rain or fog.

In North America, red spruce (Picea rubens Sarg.) decline has been observed over the past 25 years in the Appalachians under circumstances indicative of stress related disease (Siccama et al., 1982; Johnson, 1983; Vogelmann and Rock, 1988). Jagels (1986) described red spruce decline in Maine and showed that decline symptoms occurred most frequently on trees growing on thin mountain soils located on granite bedrock where ozone levels are as high as 0.14 ppm and fog pH is as low as pH 2.9. Weinstein et al. (1987) also suggested that red spruce decline in the northeastern U.S.A. may be linked to ozone and acid deposition.

Regions of southern and central Ontario have been impacted by acid rain for the past several decades (Mukammal et al., 1982; Chan et al., 1983; Heidorn and Yap, 1986; Linzon, 1987). Although Ontario is at the northern limit of the range of red spruce, white spruce (Picea glauca (Moench) Voss), is common throughout most of the province. The decline of spruce would have major economic consequences in Ontario. Direct damage to the foliage is unlikely; however, acidification of soil to within the aluminum buffering range, pH <4.2, may not only result in the loss of nutrient cations but also mobilize aluminum which has been shown to be toxic to spruce roots. Nosko et. al. (1988) and Hutchinson et. al.(1986) have found that white spruce seedlings are sensitive to aluminum. Percy (1986) found both red and white spruce seedlings sprayed with simulated acid rain of pH 4.6 had significant reductions in the number of needles compared with seedlings sprayed with pH 5.6 rain.

There is concern that increased soil acidification from acidic precipitation may inhibit the growth and establishment of white spruce on Ontario soils. In response to this concern, a mobile rain exclusion canopy system (REC) was utilized, from May 1987 to August 1989, to determine the effect of simulated acid rain (SAR) on the growth of white spruce seedlings potted in mineral soil in the presence of ambient air pollutants such as ozone.

MATERIALS AND METHODS

Mobile Rain Exclusion Canopy (REC) System

Tree seedlings were exposed to SAR in three separate treatment areas (7.6m x 15.2m) situated at the Ontario Ministry of the Environment Phytotoxicology laboratory in Brampton, Ontario. During ambient rain events, rainfall was excluded from the treatment areas by three mobile greenhouse canopies (4.6m high, 9.1m wide and 19.5m long) of a mobile rain exclusion canopy (REC) system controlled by a micro-computer (Apple IIE) and data acquisition system (Cyborg Isaac 91A). A detailed description of the REC system is given in Kuja et al. (1986).

The treatment area under each canopy was divided into two equal sections. The white spruce seedlings were placed in the northern treatment section. Each section was divided into 25 (1.5 m x 1.5 m) plots. SAR was applied to each plot in a treatment area via a nozzle mounted over the center of each plot (Figure 1).

Soil Requirements

Three soils were used in the study (Table 1). The first, referred to here as Soil 1, was collected using heavy construction equipment from a site on Margret Lake Rd. (Lot 1, Concession A, Ridout Twp.) This site is located south of Dorset, Ontario, approximately 1 km south of the Ministry of Natural Resources Leslie Frost Centre on Hwy. 35. The soil was a mixture of the B and C horizons of a humo-ferric podzol. The second soil, Soil 2, was collected by hand in May 1987 from the same site as Soil 1 but only B horizon soil was collected. The third soil, Soil 3, was collected by hand (5-30 cm depth) from a logged area located along an access road leading from Hwy 35 to Plastic Lake near Dorset, Ontario in June 1987. This soil was also the B horizon of a humo-ferric podzol.

Plant Culture

White spruce seedlings were obtained from the Ontario Ministry of Natural Resources Midhurst nursery (3+0 stock) in May 1985 and planted into 16 litre pots containing Soil 1. These seedlings were kept in pots on the ground in outdoor establishment plots and watered with tap water until the spring of 1987. A total of 450 seedlings were selected from 1500 seedlings on the basis of uniform height (30 cm) and a single leader. At this time the potted seedlings were moved into the REC treatment plots. A second set of seedlings, also from the Midhurst nursery (3+0 stock), was transplanted into 16 litre pots containing either Soil 2 or 3 in May 1987 and put directly into the treatment plots. Thus six white spruce seedlings were placed under each nozzle, two from each of the three soils (Figure 2). The pots were buried to within 3 cm of their rims. Red pine bark chips were spread around all pots to prevent soil splashing onto test plants during rain applications and to control weed growth.

Treatment Chemistry and Application

The mobile rain exclusion canopy (REC) system was designed for the application of up to five SAR treatments simultaneously. In this study, three SAR treatments were utilized in order to increase plant replication for statistical purposes. A pH 4.3 SAR treatment was selected (50 ueq $H^+ L^{-1}$) which represents the annual weighted mean pH of ambient rainfall in the southern Ontario region (Chan et. al. 1983). A more acidic pH 3.2 SAR treatment (650 ueq $H^+ L^{-1}$) was chosen to investigate the potential effect of increased rain acidity on tree growth. A pH 5.6 SAR treatment (12 ueq $H^+ L^{-1}$) was included for control purposes. In each of the three treatment areas 10 plots received pH 3.2 and 4.3 simulated acid rain and 5 plots received pH 5.6 rain solution.

Simulated acid rain (SAR) treatments were prepared by the addition of H_2SO_4/HNO_3 acids and mineral salts to deionized water (Table 2). The mineral salts were added in concentrations which approximated the average ambient rain chemistry in southern Ontario. SAR was applied on Monday, Wednesday and Friday of each week during three 10 mm events (30 mm per week). Nozzle flow rate was 0.4 mm min^{-1}. The duration of each 10 mm SAR event was 25 minutes.

During the month of June, 1987, the initial year of the study, deionized water was applied to plots to maintain soil moisture and allow tree seedlings to establish. SAR applications commenced on July 20 and were continued until October 23. In the second year, 1988, SAR was applied between the period May 10 to October 28. In the third and final year of the study, 1989, SAR was applied from May 15 to July 28. The experiment was terminated at this time.

Meteorology and Air Quality

Ambient O_3, SO_2 and NO/NO_2 were monitored daily at the study site using Monitor Labs Inc., Model 8810 photometric O3 analyzer, Teco Inc. Model 43 pulsed fluorescent SO2 analyzer and a Model 14B/E chemiluminescent NO2/NO/NOx analyzer. Temperature, relative humidity, wind velocity and photosynthetic photon flux density were measured by sensors located on an 8m tower attached to the system control trailer. The occurrence of ambient precipitation was detected with an electronic rain sensor grid (Sangamo Ltd.). The three greenhouse shelters moved automatically over treatment areas within 50 seconds whenever rain was detected by the sensor. Rainfall quantities were measured with a tipping-bucket rain gauge (Weathertronics, Model 6010) situated on the trailer roof. Half-hour means of all meteorological, air quality and precipitation data were recorded both on computer disk and paper print out.

Thermohygrographs were placed in Stevenson screens centred in each treatment area and provided a continuous measurement of temperature and humidity at all times. Modifications to micro-climate during periods when the canopies were over the plots could be detected in this way. Soil water potential was monitored by tensiometers distributed randomly in each treatment area in 1987 and again in 1989.

Measurements and Observations

Physical measurements of plant height (cm), stem diameter (mm) 5 cm above soil grade, were made monthly during each of the three growing seasons. Foliar injury was assessed throughout the experiment. Whole seedlings were harvested at the end of the experiment, in August 1989, for dry weight determination. Current year foliage was collected in August 1988 for analysis of nitrogen, phosphorus, potassium, calcium, magnesium, sulphur, manganese, zinc, iron, and aluminum. The methods used are given in the Ontario Ministry of the Environment Handbook of Analytical Methods (1983). Pigment analysis was carried out on foliage collected in September 1988 from seedlings grown on Soil 1. The pigments were initially extracted with methanol, followed by chloroform, separation by centrifugation and analysis on a HPLC. Current year needles were analyzed for chlorophyll A and B, B-carotene, antheraxanthin, violaxanthin and lutein.

Photosynthetic measurements were made on Soil 1 seedlings on days with uniform light conditions using a LICOR 6200 portable photosynthesis meter. Measurements were always made on the same current years growth, selected from the second whorl of branches from the top of each seedling. Each measurement was made for 20 seconds in a LICOR one quarter litre leaf chamber. Measurements were always taken one day after SAR was applied and under uniform light conditions.

Statistical Analyses

The slope of the plant height and stem diameter growth curves was calculated over the period from May until the end of July for 1988 and 1989. The slopes were analyzed as a randomized complete block design. The final height and diameter measurements made at the end of July 1989 were also analyzed as a randomized complete block design with the initial height or diameter (June 1987) used as the covariate. Foliar elemental analysis and shoot and root weight were analyzed as randomized complete block designs. Photosynthesis was analyzed as a repeated measures design, since the same shoot on the same plant was used for each measurement date.

RESULTS

Rain Events and Air Quality.

A summary of ambient rain events, SAR applications and air quality during the 1987, 1988 and 1989 field seasons is shown in Table 3.

In 1987, the initial year of the study, a total of 247 mm of ambient rain fell in 45 events during the treatment application period (July 20 to October 23). During this time, a total of 400 mm of SAR was applied in 40 predetermined events (three times per week). This amount approximated the long term rainfall average for the study area. In contrast, the total ambient rainfall during the field season was below average, especially during the month of August.

In the second year of the study, SAR treatments commenced on May 10 and continued until October 28, 1988. A total of 700 mm of SAR was applied in 70 events compared with 397 mm of ambient rainfall which occurred in 80 events during the same period. Again, the total amount of ambient rain was lower than the long term summer average for the area. Drought conditions prevailed through most of the month of June, although the frequency of ambient rain events was high during the months of July and August compared with 1987.

In the third and final year, treatments were applied between May 15 to July 28. During this 11 week period, 320 mm of SAR was applied in 32 events. A total of 233 mm of ambient rainfall occurred in this time in 27 events. No ambient rainfall occurred between June 24 to July 20, 1989.

Ambient ozone levels were much higher in 1988 than in 1987. The 1988 seasonal 12 hour mean ozone concentration was 0.028 ppm compared with 0.023 ppm and 0.021 ppm in the 1987 and 1989 field seasons respectively. The 1988 seasonal peak of 0.156 ppm (July 6) was also considerably higher than either the 1987 peak of 0.101 ppm or the 1989 peak of 0.91 ppm.

Measurements and Observations:

Figure 3 shows the pattern of height increase of white spruce seedlings grown in Soil 1. The growth patterns for seedlings grown in Soils 2 and 3 were virtually identical to that of Soil 1 so are not shown. Figure 4 gives a comparison of the height increase at the time of harvest for all three soils. There was no significant difference among treatments for any one soil. The greater increase in height of the seedlings grown in Soil 1 can be attributed to the extra year of acclimatization of these seedlings. Height growth was poor in 1987 and

1988 but showed a dramatic increase in 1989. The final height of Soil 1 seedlings was approximately 82 cm and the final heights of the seedlings grown in Soils 2 and 3 were 58 and 54 cm respectively.

Figure 5 illustrates the pattern of stem diameter increase of white spruce seedlings grown in Soil 1. The growth patterns for seedlings grown in Soils 2 and 3 were virtually identical to that of Soil 1 as was the case for height increase. Stem diameter increased steadily during the growing season from July 1987 to July 1989. Figure 6 shows the stem diameter increase at the time of harvest for all three soils. There were no significant differences among treatments for any one soil. The greater stem diameter increase of the seedlings grown in Soil 1 can be attributed to the extra year of growth of these seedlings. The Soil 1 seedlings show a trend of increasing stem diameter with increasing pH. The seedlings grown in Soils 2 and 3 show the opposite trend.

The dry shoot weight of the seedlings, at the time of harvest in August 1989, and grown in Soil 1, is given in Figure 7. The dry weights of both roots and shoots of seedlings grown in Soils 2 and 3 are shown in Figure 8. There was no significant difference due to treatment among either shoot or root weights. There was a tendency for the shoots to have a greater biomass in the more acidic treatments compared to the pH 5.6 treatment. Root weight was apparently unaffected by the differences in levels of acidity in the spray solutions.

The photosynthetic rate of white spruce seedlings grown in Soil 1 is shown in Figure 9. The plants sprayed with pH 3.2 SAR had consistently lower photosynthetic rates than the other treatments. Statistical analysis using the repeated measures procedure failed to show any statistical significance to this trend. The light conditions ranged from 412 to 1945 umol m^{-2} s^{-1} but the photosynthetic rate remained relatively constant between 0.03 and 0.04 umol CO_2 m^{-2} s^{-1}.

A sample of current year needles from these plants collected in September 1988 and analyzed for pigments showed no significant differences in pigment concentrations due to treatments or soil. The following mean values of the pigments were considered to be representative of the pigment concentrations in the foliage; chlorophyll a 4427 ug/g, chlorophyll b 1470 ug/g, B-carotene 56 ug/g, antheraxanthin 124 ug/g, lutein 446 ug/g and violaxanthin 148 ug/g.

Current-year needles from all white spruce seedlings in all soils were analyzed for nitrogen, phosphorus, potassium, sulphur, calcium, magnesium, iron, manganese, and aluminum (Table 4 to 6). The only significant treatment effects were in manganese concentrations in Soil 1 and sulphur concentrations in Soil 3. Manganese concentrations were higher in pH 5.6 foliage compared to concentrations in pH 4.3 or 3.2 foliage and sulphur concentrations were higher in the pH 3.2 treatment compared to pH 4.3 or 5.6. All other foliar elemental concentrations were similar for the three acidity levels, regardless of the soil in which the seedlings were grown.

DISCUSSION

In this experiment, three mineral soils were collected from an area of Ontario impacted by acid rain and having sensitive soils. A soil with high sensitivity is defined as having a pH < 5.5, if a sandy soil, a low cation exchange capacity, and non-carbonate bearing parent material. A non-sensitive soil is defined as a soil with a pH > 5.5, a high cation exchange capacity and a carbonate parent material (Cowell 1986). Cowell (1986) estimates that 31.4% of Ontario soils are highly sensitive and 23.1 % are non-sensitive. Using this classification Soils 1 and 2 were non-sensitive, whereas Soil 3 is sensitive. None of the three soils used are within the aluminum buffering range of pH <4.2. It was anticipated that Soil 3, with a pH of 5.1 (water extr.), might be acidified through several years of acid rain applications to within the aluminum buffering range. In addition, the soil material collected was primarily from the B horizon in Soil 1, and entirely B horizon material in Soils 2 and 3. The B horizon has the highest concentration of iron and aluminum and as a result was considered to be the horizon most likely to produce symptoms of aluminum toxicity under acidic conditions. There was, however, no measurable change in the pH of the soil over the experimental period and no evidence of aluminum toxicity either in terms of root damage or foliar concentration (Table 4 to 6).

Mineral soil is considered to be a good seed bed for white spruce (Arnup et al. (1988). Texturally, Soil 3, was a finer soil with a much higher percentage of silt than Soils 1 or 2. Since sandy silt is more suitable for spruce growth than sand (Arnup et al. 1988), one might expect better growth in Soil 3. Growth was actually poorest in Soil 3 which is probably more a reflection of the nutrient status of the soil than its texture. The white spruce seedlings in Soil 3, as well as Soil 2, may have experienced transplant shock also.

The pattern of growth among all soils (Figure 3) was virtually identical in spite of the fact the trees in Soil 1 were one year older and had been transplanted into pots one year before the trees in the other two soils. The results suggest that the Soil 1 spruce seedlings were stressed in the establishment plots and required two years in the REC system to start rapid growth. The lack of growth in height in the first year or two after transplantation is common in white spruce. This "check" is often due to a general shock to the plant, damage to the root system with a consequent alteration in the root:shoot ratio (Sutton 1990) or nutrient stress (Sutton 1968). The sandy nature of the soil and low nutrient status exacerbated the damage due to transplanting. This result also underlines the need for long term experiments. Short term experiments with transplanted spruce seedlings cannot realistically assess the impact of the acid rain stress since the overwhelming stress is due to transplanting.

It was anticipated that 'spruce seedlings grown in the most acidic soil, Soil 3, would show a general decrease in growth with increased acidification and the least sensitive soil, Soil 1, little effect or even an increase in growth. Ashenden and Bell (1988) reported a similar response in Sitka spruce on a range of British soils. This clearly was not the case in the REC experiment. Seedlings grown in the least sensitive soil, Soil 1, showed a trend toward a growth decrease in the most acidic treatment. Ulrich (1983) has pointed out that soil acidification comparable to podzolization can be found below the stem base of beech trees on limestone soil in Germany, a soil which would be non-sensitive according to the criteria of Cowell (1986). He argues that the acidic stemflow is funnelled down the stem and into the soil around the tree base and root surfaces resulting in the acidification of this area. This acidification did not happen in the REC experiment, since the spruce foliage tended to deflect the acid rainfall away from the stem base and no change in soil pH was measured.

The reason that spruce seedlings grown on Soil 1 show the greatest response to the acid rain treatments may be because the soil was a coarse sand into which the acid rain solutions would rapidly infiltrate. As a consequence of this rapid infiltration the roots may have been subjected to pulses of acidity. Soil 3 with its finer texture may have had slower infiltration and greater interaction between the soil particles and the rain solution resulting in less of an acid pulse. Although these trends in growth were not statistically significant, alterations in soil chemistry which may cause damage would take a much longer period of time than the two and one half years over which this experiment was conducted.

Seedlings grown in Soils 2 and 3 showed the greatest growth in the pH 3.2 treatment (Figure 4). McLaughlin et al. (1988) report a slight stimulation in the height growth of Loblolly pine exposed to pH 3.2 SAR. Similarly, Ashenden and Bell (1988) found that Sitka spruce seedlings exposed to pH 3.5 rain had generally greater growth than seedlings exposed to either pH 4.5 or 5.6 simulated acid rain. The greater concentration of nitrate and sulphate in the pH 3.2 SAR compared to the pH 5.6 SAR may have had a slight fertilizing effect and may explain the greater growth in this treatment (Stuanes 1983).

The rate of stem diameter increase was consistent among all three soils and among the three treatment regimes. There was no indication of check due to transplantation, nor was there a spring surge in growth followed by a levelling off in mid summer as had been evident in height growth. Even under stress conditions the seedling produces new cambial tissue each year whereas it is not essential that the tree increase in height. In the initial period of establishment after transplanting, photosynthate is primarily allocated to the root system rather than the shoots. This pattern of allocation may occur for years until sufficient root system is established to support shoot growth. The rapid growth in 1989 indicated that the spruce roots had established sufficiently to support rapid shoot growth. Increase in stem diameter was constant. Simulated acid rain treatments had no effect on stem diameter or root weight.

Reports of damage to spruce caused by simulated acid rain in controlled experiments have often resulted from the use of extremely high concentrations of acidity in the sprays. Weinstein et al. (1987) found acid mist of pH 2.5 caused significant injury to needles of two populations of red spruce. Percy (1986) investigated the effect of SAR on 11 tree species including white spruce. Adverse effects were noted on all parameters measured at pH 2.6 but only significant decreases were noted in cotyledon length and primary needle initiation at pH 3.6. Percy (1986) applied sprays twice a day, every day for 5 weeks. In spite of this drastic spray regime, very few adverse effects were noted at either pH 4.6 or 3.6, which are within the range of ambient events.

McLaughlin et al. (1988) conducted a large field and laboratory experiment on the response of loblolly pine genotypes to ozone and acid deposition. Acid rain caused a general decrease in height and diameter growth at the highest level of acidity, pH 3.3, and a general stimulation in growth at pH 4.3. These results were not statistically significant. The Ministry of the Environment experiment was designed to investigate the effects of acid rain on white spruce seedlings within the range of acidities which occur in southern Ontario. Acidic rain below pH 3.0 were not used since such regimes were considered unnaturally low for Ontario. The pH 3.2 treatment used was highly acidic and yet, as with the experiments of McLaughlin et al. (1989), produced no significant effects on growth.

The duration of an experiment can have a major effect on the results. Short term experiments are often unable to detect subtle changes which in time may lead to major effects. Laurence et al. (1989) exposed one year old red spruce seedlings to SAR of pH 3.1, 4.1 and 5.1 as well as a range of ozone treatments. They found no treatment effects due to simulated acid rain but added the caveat that it was the first year of a three year study and that "effects of simulated rain chemistry might be expected to be greater during the primary growth phase." The Ministry of the Environment experiment lasted two and one half years but showed no significant growth effects due to acid rain.

The genetic material used in an experiment can have a major effect on the study results. McLaughlin et al. (1988) investigated the effects of ozone and acid rain on 52 half sibs of loblolly pine and found significant genotype-pollutant interactions. The 450 seedlings used in the REC experiment were selected from several thousand seedlings on the basis of uniform height (30 cm) and single leaders. Seedlings which were damaged or in poor condition were not used. Nevertheless there was a great deal of variability in individual response to the treatments applied which in part may reflect genetic differences. The use of a genetically diverse population increases the variability in the experiment but the response is more representative of seedlings growing in central Ontario.

Bergmann and Scholz (1987) demonstrate that genetic changes will occur in Norway spruce if the sensitive trees die prior to reproduction. They observed that trees tolerant to O_3, SO_2 and NO_2 exhibited a generally higher genetic diversity than sensitive trees but caution that with the death of sensitive trees there will be a potential loss of rare alleles. Bormann (1985) raises the point that genetically based resistance to air pollution may have hidden costs like lowered productivity and loss of fitness. This may have repercussions in the ability of trees to cope with other stresses such as climate change.

The dry weight of the roots of seedlings grown in Soils 2 and 3 were taken as a crude measure of root growth. Changes in root growth are critically important to a seedling's survival and are integrally linked to the seedling's water and nutrient status. Figure 8 shows virtually identical root weights among treatments and between Soils 2 and 3. Since the shoots tended to be slightly larger in the pH 3.2 treated seedlings, the root to shoot ratio was smaller in this treatment but this had no effect on seedling vigor.

Photosynthesis measurements were recorded for Soil 1 seedlings in order to determine short term stress attributable to acid precipitation. The pH 3.2 sprayed seedlings consistently had lower photosynthetic rates than the pH 4.3 or 5.6 sprayed plants. Although these results were not statistically significant they indicate a potential impact of the highest acidity rainfall on plant photosynthesis. Interestingly, the above trend was also evident in height and stem growth in Soil 1 and in the dry weight of the shoots. Continuation of this trend in a longer term study could ultimately lead to significant differences in growth.

In this experiment the trend toward increased growth in Soils 2 and 3 indicates a nitrogen and sulphur fertilizer effect. In eastern Canada, nitrate is an increasingly important component of acid deposition since concentrations in rainfall are increasing while concentrations of sulphur have been decreasing over the past 15 years (Tang et al. 1990). Therefore, nitrate loading may become increasingly important to the terrestrial ecosystem in eastern Canada. The most acidic treatment utilized in the REC system gave a loading of 32 kg ha^{-1} yr^{-1} and 3.2 kg ha^{-1} yr^{-1} for nitrate and ammonium respectively. These loadings are very high but are similar to those reported by Bruck et al. (1989) of 25.3 kg ha^{-1} yr^{-1} and 9.5 kg ha^{-1} yr^{-1} of nitrate and ammonium for an Appalachian site experiencing a decline of red spruce and Fraser fir. Schulze (1989) suggests that since the 1960s, in Europe, nitrate deposition has been the major cause of soil acidification. Schulze (1989) states that, prior to the 1960's, nitrate inputs had a fertilizer effect. Although the loading in eastern Canada is not as high as in Europe, and the soils tend to be low in nitrogen, the long term effects may be soil acidification, similar to that being experienced in Europe.

Stuanes (1983) and Friedland et al. (1985) have suggested that nitrogen deposition can lead to a stimulation in plant growth which, if late in the season, could lead to decreased winter hardiness. The nitrogen input from the more acidic SAR treatments (pH 3.2, 4.3), over the course of this two and one half year experiment, did not cause a significant increase in growth of the white spruce seedlings compared to the pH 5.6 treated seedlings, nor was there any indication of greater seedling mortality or needle necrosis due to increased nitrogen input. Recent research has established that nitrogen inputs are unlikely to be the cause of winter kill and that they can actually increase the tolerance of red spruce to winter injury (DeHayes et al. 1989, Klein et al. 1989).

Nutrient concentrations in the three soils varied considerably yet were generally low (Table 4 to 6). The foliar concentrations of nitrogen in seedlings grown on Soils 2 and 3 ranged from 1.1 to 1.2 % compared to 1.5 to 2.1% for 3+0 white spruce seedlings from southern Ontario forest tree nurseries (Armson and Sadreika 1974). Foliage from Soil 1 spruce had higher nitrogen concentrations, ranging from 1.8 to 2.0%. Similarly foliar phosphorus concentrations, in seedlings grown in Soils 2 and 3, were approximately 0.11 % compared to a range of 0.10 to 0.23% for 3+0 seedlings (Armson and Sadreika 1974). Soil 1 seedlings had a higher foliar phosphorus concentration of 0.16%. Foliar concentrations of potassium were slightly lower in seedlings from all Soils (0.39 to .49%) compared to those given by Armson and Sadreika (1974) of 0.59 to 1.20%. These results indicate that all seedlings were grown under nutrient poor conditions and that seedlings grown in Soils 2 and 3 were suffering from nitrogen and phosphorus deficiencies. It is often assumed that acidic rain will add another stress and therefore have the greatest effect on plants already stressed. This was not the case since growth was greatest in the most acidic treatment, pH 3.2.

Foliar leaching of nutrients, such as potassium, due to acid rain has been thought to be a potential problem. Joslin et al. (1988) report elevated levels of K, Ca, Mg, Na and SO_4 in throughfall from a red spruce stand and suggest that this loss of nutrients might be a problem on nutrient poor soils. Kuja and Dixon (1987) have found increased foliar leaching of potassium from white spruce foliage sprayed with simulated acid rain in the greenhouse. However, even if potassium is leached from the foliage, it is effectively cycled back through the roots and up into the foliage again. Stone and Kszystniak (1977) report that stands of

Pinus resinosa fertilized with a single application of potassium derived 60% of the foliar potassium from the fertilizer 9 years after the application and 40%, 23 years after the application. They report similar responses in white and red spruce. In the REC experiment foliar leaching may have occurred, however, foliar analysis (Table 6) gives no indication that the foliar concentrations of any of the major nutrients, N, P or K, were affected by the acid rain treatments (Table 4 to 6).

Two elements showed significant differences in foliar concentrations due to the SAR treatments (Table 4 and 6). Manganese concentrations in seedlings exposed to pH 5.6 rain in Soil 1 were higher than those exposed to the pH 3.2 rain solutions (Table 6). Manganese is readily displaced by other metals and, therefore, it is common for manganese concentrations in plant tissue to decrease as concentrations of other metals such as iron, copper, zinc, increase (Rinne and Barclay-Estrup 1980). Acid deposition may increase the mobility of metals in the soil which can lead to greater plant uptake and greater displacement of manganese. Zinc, iron and aluminum were not elevated in the pH 3.2 treated foliage.

The sulphur concentrations in the foliar of seedlings in Soil 3 exposed to simulated acid rain at pH 3.2 were higher compared to the pH 5.6 treatment (Table 6). This reflects the higher sulphate concentrations in this treatment. Sulphur concentrations were highest in the pH 3.2 treated plants in all soils, although, in Soils 1 and 2, this was not statistically different ($p<.05$).

CONCLUSION

SAR applications to white spruce seedlings for two and one half growing seasons did not produce any significant treatment effects on growth, nutrition or physiology. There was no measurable change in the pH of the soil and no evidence of aluminum toxicity. Foliar sulphur and nitrogen concentrations tended to be highest in the pH 3.2 treated seedlings in all soils. Sulphur concentrations were significantly higher in Soil 3. This difference is attributable to the higher sulphur and nitrogen inputs in the pH 3.2 SAR compared to either the pH 4.3 or pH 5.6 and may account for a tendency toward greater growth in seedlings receiving this treatment. Photosynthesis tended to be lowest in the pH 3.2 treatment but this was not reflected in any measure of growth. None of the foliar pigments analyzed were significantly altered due to the treatment applied. The evidence of growth check in the spruce seedlings for the first two years after transplanting indicates that long term studies are required in order to assess the effects of pollutants on growth of white spruce.

The lack of response of the white spruce seedlings to the SAR treatments suggests that white spruce seedlings in Ontario are unlikely affected by ambient acidic precipitation. However, long term alterations in soil chemistry due to atmospheric deposition of pollutants may result in a growth reduction in this species and should be the objective of longer term studies.

REFERENCES

Armson, K.A and V. Sadreika. 1974. Forest tree nursery soil management and related practices. Ontario Ministry of Natural Resources.

Arnup, R.W., B.A. Campbell, R.P. Raper, M.F. Squires, K.D. Virgo, V.H. Wearn and R.G. White. 1988. A silvicultural guide for the spruce working group in Ontario. Ontario Ministry of Natural Resources, Forest Resources Group.

Ashenden, T.W. and Bell, S.A. 1988. Growth responses of birch and sitka spruce exposed to acidified rain. Environ. Pollut. 51: 153.

Bergmann, F. and F. Scholz. 1987. Selection effects of air pollution in Norway spruce (Picea abies) populations. In: Genetic Effects of Air Pollutants in Forest Tree Populations F.Scholz, H.-R. Gregorius and D. Rudin (eds.) Springer-Verlag, Berlin.

Bormann, F.H. 1985. Air pollution and forests: an ecosystem perspective. Bioscience 35: 434.

Brand, D.C. and Janas, P.S. 1988. Growth and acclimation of planted white pine and white spruce seedlings in response to environmental conditions. Can. J. For. Res. 18: 320.

Bruck, R.I. W.P. Robarge and A. McDaniel. 1989. Forest decline in the boreal montane ecosystems of the southern Appalachian mountains. Water, Air and Soil Pollut. 48: 161.

Chan, W.H., A.J.S. Tang and M.A. Lusis, 1983. Precipitation concentration and wet deposition fields of pollutants in Ontario September 1980 to December 1981. Report ARB-61-83-ARSP Ontario Ministry of the Environment, Toronto, Canada.

Cowell, D.W. 1986. Assessment of aquatic and terrestrial acid precipitation sensitivities for Ontario. Report ARB-220-86-Phyto, Ontario Ministry of the Environment, Toronto, Canada.

DeHayes, D.H., M.A. Ingle and C.E. Waite. 1989. Nitrogen fertilization enhances cold tolerance of red spruce seedlings. Can. J. For. Res. 19: 1037.

Friedland, A.J., G.J. Hawley and R.A. Gregory. 1985. Investigations of nitrogen as a possible contributor to red spruce (Picea rubens Sarg.) decline. In: Proceedings of the Symposium on the Effects of Air Pollution on Forest Ecosystems, Minneapolis, Minnesota, May 8-9, pp. 95-106.

Heidorn, K. and D. Yap, 1986. A synoptic climatology for surface ozone concentrations in southern Ontario 1976-81. Atmos. Environ. 20: 695.

Hornbeck, J.W. and R.B. Smith, 1985. Documentation of red spruce growth decline. Can. J. For. Res. 15: 1199.

Hutchinson, T.C., L. Bozic, G. Munoz-Vega, 1986. Response of five species of conifer seedlings to aluminum stress. Water, Air, Soil Pollut. 31: 283.

Jagels, R., 1986. Acid fog, ozone and low level spruce decline. IAWA Bull. 7: 299.

Johnson, A.H., 1983. Red spruce decline in northeastern U.S.A.: hypothesis regarding role of acid rain. J. Air Pollut. Cont. Assoc. 33: 1049.

Joslin, J.D., Kelly, J.M. and Wolfe, M.H. 1988. Elemental patterns in roots and foliage of mature spruce across a gradient of soil aluminum. Water, Air and Soil Pollut. 40: 375.

Klein, R.M., T.D. Perkins and H.L. Myers. 1989. Nutrient status and winter hardiness of red spruce foliage. Can. J. For. Res. 19: 754.

Kuja, A.l., R. Jones, A. Enyedi, 1986. A mobile rain exclusion canopy system to determine dose-response relationships for crops and forest species. Water, Air, Soil Pollut. 31: 307.

Kuja, A.L. and M.J. Dixon. 1987. Five year study using a mobile rain exclusion canopy system to determine joint effects of simulated acid rain and ozone on the growth and physiology of sugar maple and white spruce seedlings. In: Proceedings of the Technical Transfer Conference, Part A, Air Quality Research, Royal York Hotel, Toronto, Nov. 30 to Dec. 1, 1987.

Laurence, J.A., R.J. Kohut and R.G. Amundson. 1989. Response of red spruce seedlings exposed to ozone and simulated acidic precipitation in the field. Arch. Environ. Contam. Toxic. 18: 285.

Lefohn, A.S., R.W. Brocksen, 1984. Acid rain effects research: a status report. J. Air Pollut. Cont. Assoc. 34: 1005.

Linzon, S.N., 1985. Forest damage and acidic precipitation IN: E.B. Eddy Distinguished Lecture Series Nov. 1985, Faculty of Forestry, Univ. of Toronto, Toronto, Ontario.

McLaughlin, S.B., M.B. Adams, N.T. Edwards, P.J. Hanson, P.A. Layton, E.G. O'Neill and W.K. Roy. 1988. Comparative sensitivity, mechanisms, and whole plant physiological implications of responses of loblolly pine genotypes to ozone and acid deposition. Publ. No. 3105. Oak Ridge National Laboratory, Environmental Sciences Division.

Mukammal, E., H. Nuemann, T. Gillespie, 1982. Meteorological conditions associated with ozone in southwestern Ontario, Canada. Atmos. Environ. 16: 2095.

Nosko, P., P. Brassard, J.R. Kramer, K.A. Hershaw, 1988. The effect of aluminum on seed germination and early seedling establishment, growth and respiration of white spruce (Picea glauca). Can. J. Bot. 66: 2305.

Ontario Ministry of the Environment 1983. Handbook of analytical methods for environmental samples. Vol. 1 and 2. Laboratory Services Branch of the Ontario Ministry of the Environment.

Percy, K., 1986. The effects of simulated acid rain on germination capacity, growth and morphology of forest tree seedlings. New Phytol. 104: 473.

Rinne, R.J.K. and Barclay-Estrup, P. 1980. Heavy metals in a feather moss, Pleurozium schreberi, and in soils in N.W. Ontario, Canada. Oikos 34: 59.

Schulze, E.-D. 1989. Air pollution and forest decline in a spruce (Picea abies) forest. Science 244: 776.

Siccama, T.G., M. Bliss and H.W. Vogelmann, 1982. Decline of red spruce in the Green Mountains of Vermont. Bull. Torrey Bot. Club 109: 162.

Stone, E.L. and R. Kszystyniak. 1977. Conservation of potassium in the Pinus resinosa ecosystem. Science 198: 192.

Stuanes, A.O. 1983. Possible indirect long-term effects of acid precipitation on forest growth. Aquilo Serie Botanica 19: 50.

Sutton, R.F. 1969. Silvics of white spruce (Picea glauca [Moench] (Voss). Publ. No. 1250. Can. Dept. Fisheries and Forestry.

Sutton, R.F. 1990. Root growth capacity in coniferous forest trees. Hort. Sci. 25: 259.

Tang, A., N. Reid and M. Lusis 1990. Temporal variability of the seasonal sulphate concentrations in precipitation at Dorset, Ontario. Ontario Ministry of the Environment (in press).

Ulrich, B., R. Mayer, P.K. Khanna, 1980. Chemical changes due to acid precipitation in a loess-derived soil in central Europe. Soil Sci. 130: 193.

Ulrich, B., 1983. Soil acidity and its relations to acid deposition. IN: B. Ulrich and J. Pankrath (eds.) Effects of Air Pollution on Forest Ecosystems. Reidel Publ.,Dordrecht, F.R.G. pp. 1-32.

Van Breemen, N., 1985. Acidification and decline of central European forests. Nature 316:6.

Vogelmann, A.F. and B.N. Rock, 1988. Anatomy of red spruce needles from forest decline sites in Vermont. Environ. and Exp. Bot. 28: 19.

Weinstein, L.H., R.J. Kohut, J.S. Jacobson, 1987. Research at Boyce Thompson Institute on the effects of ozone and acidic precipitation on red spruce. In: Proceedings of 80th annual meeting of APCA, New York, New York, June 21-26, 1987. pp. 87-341.

Zoettl, H.W. and R.F. Huettl, 1986. Nutrient supply and forest decline in southwest Germany. Water, Air and Soil Pollut. 31: 449.

Table 1. Chemistry of soils utilized in the study

	SOIL 1	SOIL 2	SOIL 3
Parameter	Leslie Frost[+] Site (5-50 cm)	Leslie Frost[#] Site (5-30 cm)	Plastic Lake[!] Site (5-30 cm)
pH (water ext.	5.6-7.5	5.7	5.1
pH ($CaCl_2$ ext.)	5.0-7.0	4.8	4.5
% Sand	90	90	37
Silt	7	7	58
Clay	3	3	5
% organic C	2.4	4.0	2.3
Ca[•] (me/100g)	7.96	1.1	0.66
Mg[•] (me/100g)	0.36	0.10	0.02
K[•] (me/100g)	0.03	0.01	0.02
Al[•] (me/100g)	0.20	0.20	0.54
Ca (mg/g)	18.4	2.54	2.2
Mg (mg/g)	3.1	0.86	1.3
K (mg/g)	4.8	0.65	0.43
Al (mg/g)	0.014	-	14.3
N (mg/g)	1.49	1.87	0.93
P (mg/g)	0.91	0.85	0.46
Fe (mg/g)	0.06	-	20.0
S (mg/g)	1.1	-	0.15
Cu (ug/g)	16	15	9
Ni (ug/g)	2	13	7
Pb (ug/g)	5	5	5
Cd (ug/g)	0.2	0.2	<.2
Zn (ug/g)	20	44	39

[+] Soil removed by heavy machinery in October 1985.

[#] Soil removed by hand in May 1987.

[!] Soil removed by hand in June 1987.

[•] NaCl extracted concentrations

Table 2. Chemical composition of simulated acidic rain (SAR) solutions.

SAR[*] pH	H^+ ueq L^{-1}	Acid vol. ml L^{-1}	
		H_2SO_4	HNO_3
5.6	12	0	0
4.3	50	1.001 E^{-3}	8.849 E^{-3}
3.2	650	1.301 E^{-2}	1.151 E^{-2}

[*] All simulated rain solutions contained the following background ions (mg L^{-1}):

K - 0.063

Mg - 0.103

Na - 0.100

Cl - 0.023

Ca - 0.501

NH_4 - 0.744

NO_3 - 1.385

SO_4 - 2.771

S:N ratio = 2:1

Table 3. Summary of simulated acid rain (SAR) and ambient rain events during the 1987, 1988, and 1989 growing seasons.

Year	Type of Event	Total number	Amount (mm)	Time canopies over plots (hr)
1987[•]	SAR[+]	40	400	16.7
	Ambient	45	247	185.0
1988[']	SAR	70	700	29.2
	Ambient	80	397	217.5
1989[@]	SAR	32	320	13.3
	Ambient	27	233	127.1

[•] 1987 - SAR was applied from July 20 to October 23.

['] 1988 - SAR was applied from May 10 to October 28.

[@] 1989 - SAR was applied from May 5 to July 27.

[+] SAR applied as three 10 mm events per week in each season.
SAR application was continuous at a rate of 0.4 mm $\text{min}^{-1.}$
Each SAR event was 25 minute duration.

Table 4: Elemental Analysis of Current Year White Spruce Needles Grown in Soil 1 and Exposed to Simulated Acid Rain

ELEMENT	pH 3.2	pH 4.3	pH 5.6
N	19000	20000	18000
P	1600	1700	1700
K	4500	4900	4800
S	610	600	610
Ca	11000	11000	10000
Mg	720	720	770
Mn	44a	46a	55b
Zn	32	36	36
Fe	84	93	90
Al	46	50	48

Note: elemental concentrations (ug/g dry weight) are not significantly different ($p<0.05$) with the exception of manganese as indicated by the letters following the number.

Table 5: Elemental Analysis of Current Year White Spruce Needles Grown in Soil 2 and Exposed to Simulated Acid Rain

ELEMENT	pH 3.2	pH 4.3	pH 5.6
N	12000	12000	11000
P	1100	1100	1000
K	4000	4000	3900
S	760	760	720
Ca	7200	7000	7000
Mg	1000	1000	1000
Mn	340	300	290
Zn	44	44	43
Fe	83	95	94
Al	79	78	78

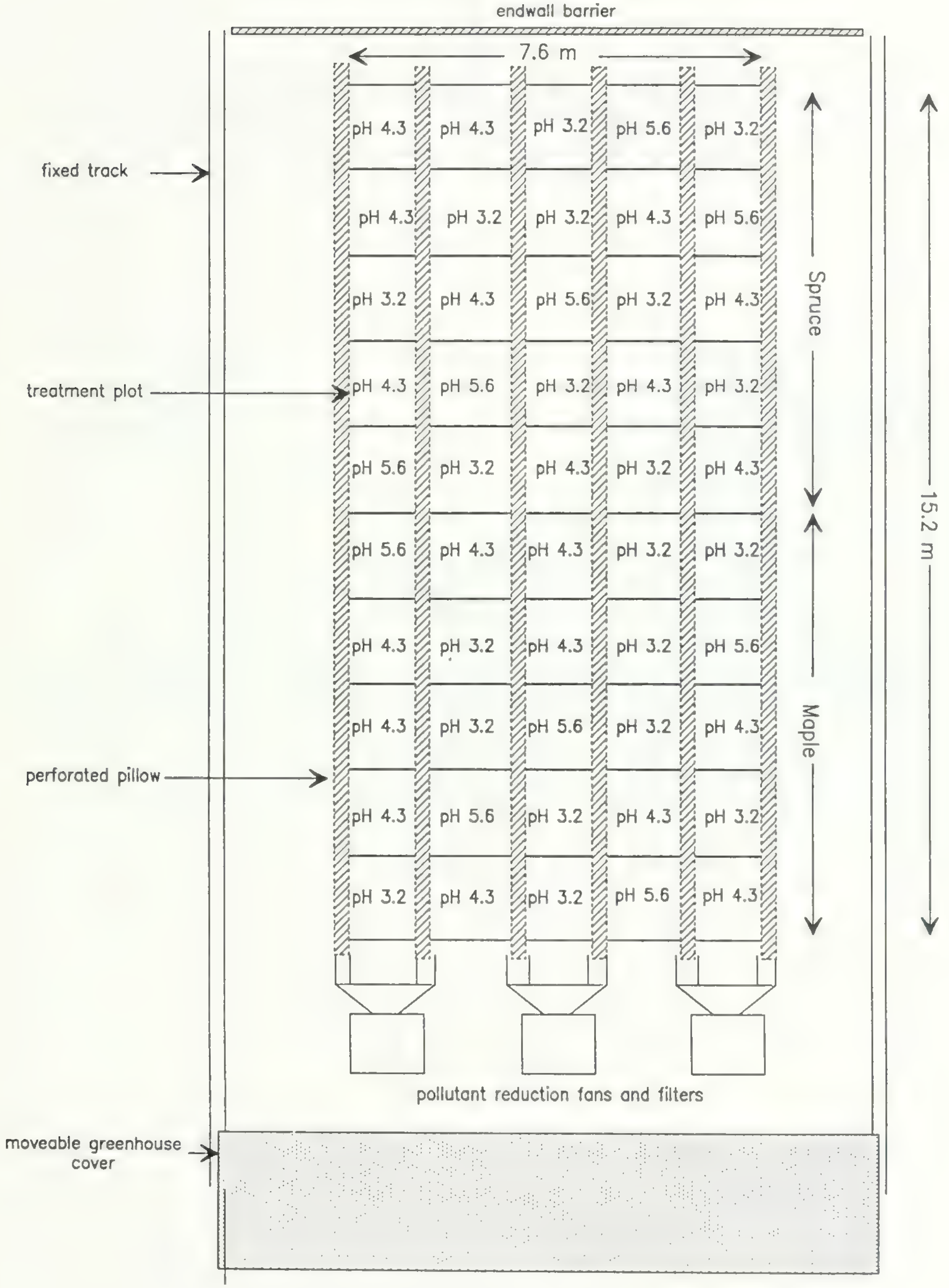
Note: elemental concentrations (ug/g dry weight) are not significantly different ($p<0.05$).

Table 6: Elemental Analysis of Current Year White Spruce Needles Grown in Soil 3 and Exposed to Simulated Acid Rain

ELEMENT	pH 3.2	pH 4.3	pH 5.6
N	12000	12000	11000
P	1100	1100	990
K	4000	3900	3900
S	660a	610ab	590b
Ca	8500	7700	7900
Mg	1000	1000	1000
Mn	580	480	560
Zn	70	64	67
Fe	100	92	104
Al	79	79	87

Note: elemental concentrations (ug/g dry weight) are not significantly different ($p<0.05$) with the exception of sulphur as indicated by the letters following the number.

Figure 1: Diagram of the Rain Exclusion Canopy System treatment plots



pH 4.3	pH 4.3	pH 3.2	pH 5.6	pH 3.2
pH 4.3	pH 3.2	pH 3.2	pH 4.3	pH 5.6
pH 3.2	pH 4.3	pH 5.6	pH 3.2	pH 4.3
pH 4.3	pH 5.6	pH 3.2	pH 4.3	pH 3.2
pH 5.6	pH 3.2	pH 4.3	pH 3.2	pH 4.3
pH 5.6	pH 4.3	pH 4.3	pH 3.2	pH 3.2
pH 4.3	pH 3.2	pH 4.3	pH 3.2	pH 5.6
pH 4.3	pH 3.2	pH 5.6	pH 3.2	pH 4.3
pH 4.3	pH 5.6	pH 3.2	pH 4.3	pH 3.2
pH 3.2	pH 4.3	pH 3.2	pH 5.6	pH 4.3

Figure 2: Plot position of white spruce seedlings potted in three mineral soils

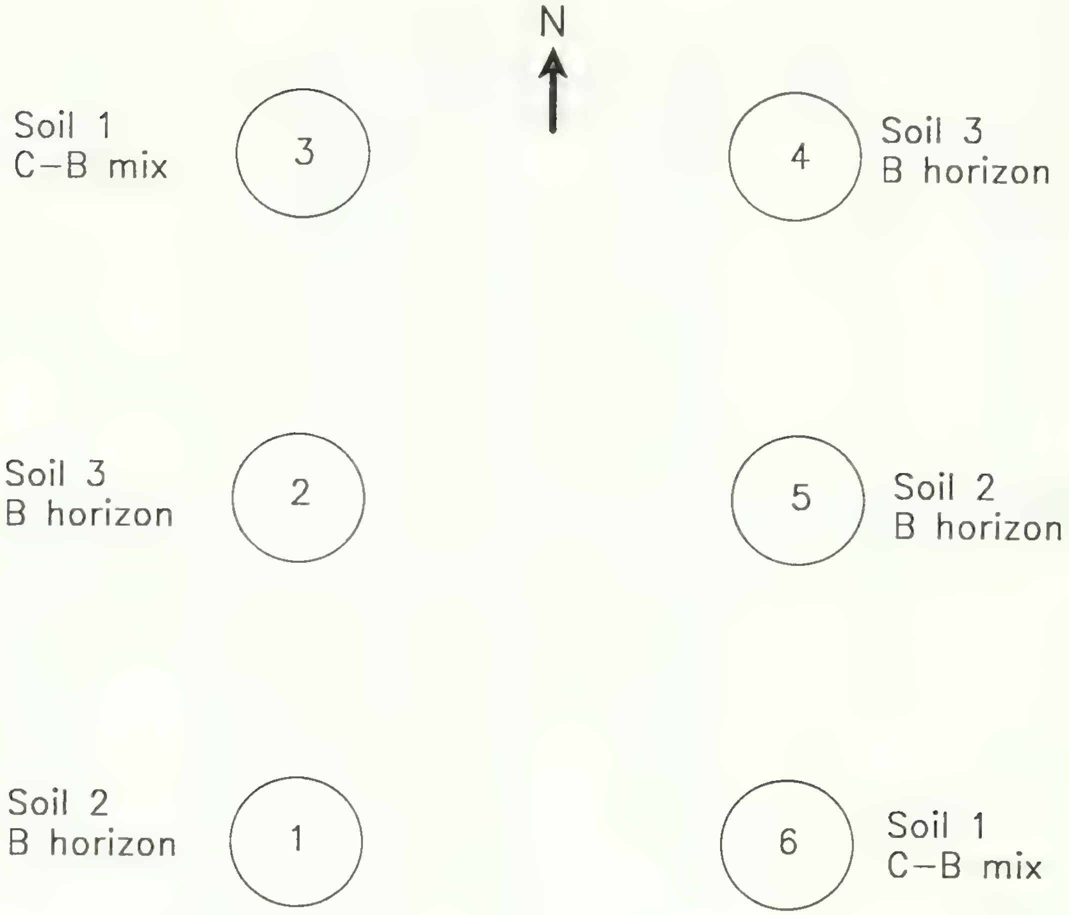


Figure 3: Height increase of white spruce seedlings grown in Soil 1 and exposed to simulated acid rain for two and one half growing seasons

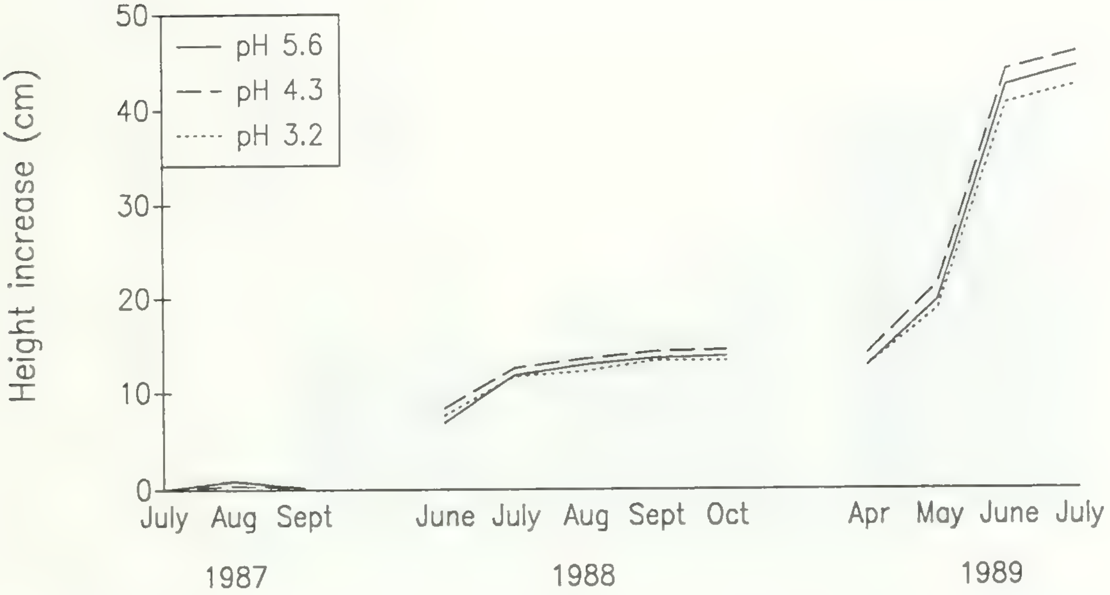
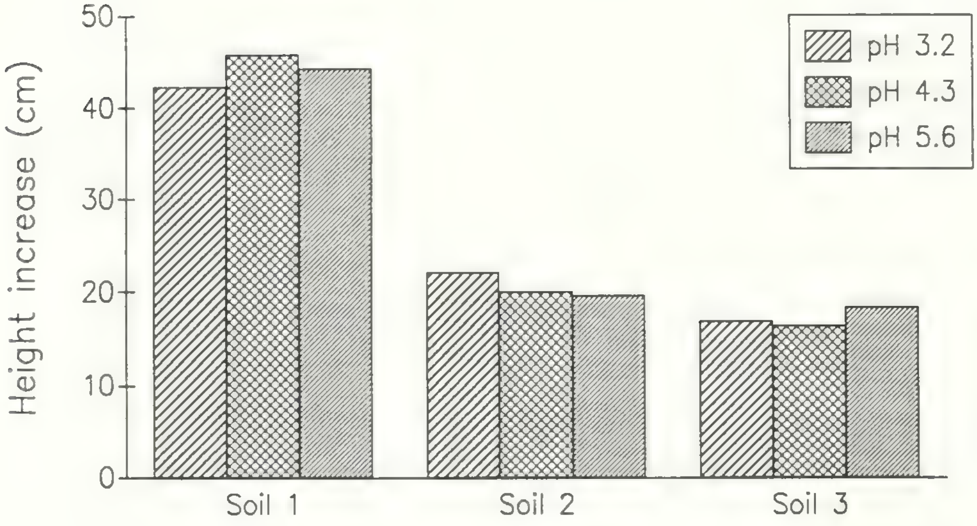


Figure 4: Height increase of white spruce seedlings exposed to simulated acid rain during the growing season from June 1987 to August 1989



treatments columns, for any soil, are not significantly different ($p < 0.05$)

Figure 5: Stem diameter increase of white spruce seedlings grown in Soil 1 and exposed to simulated acid rain for two and one half growing seasons

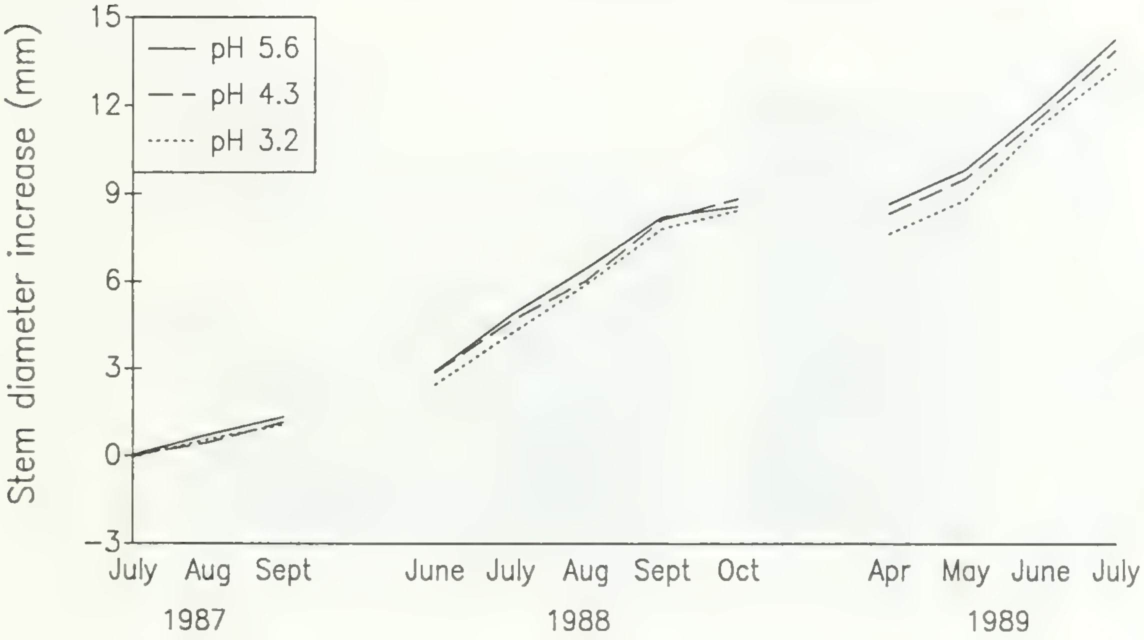


Figure 6: Stem diameter increase of white spruce seedlings exposed to simulated acid rain during the growing season from June 1987 to August 1989

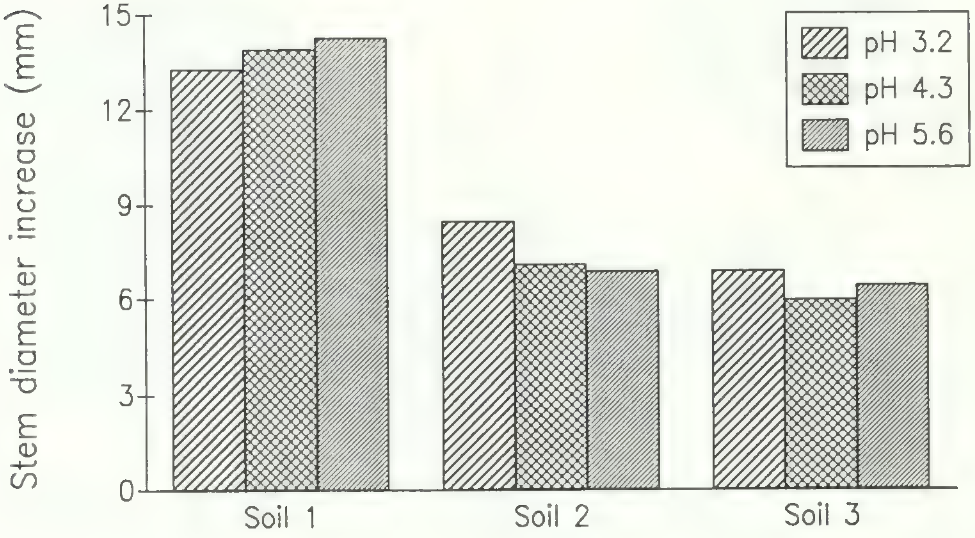


Figure 7: Dry weight of shoots of white spruce seedlings grown in Soil 1 and exposed to simulated acid rain for two and one half years

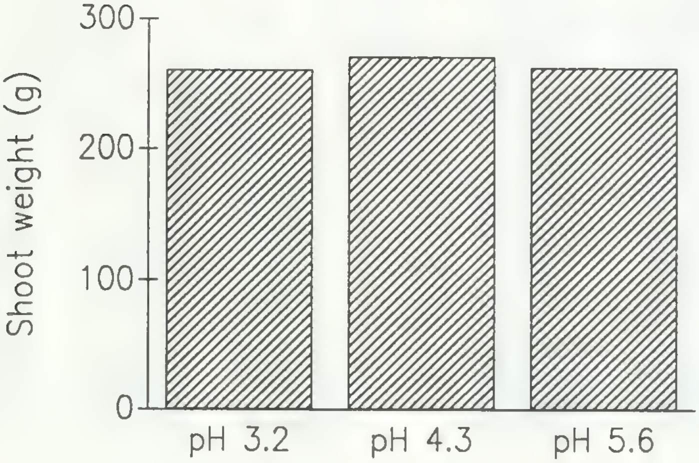


Figure 8: Dry weight of roots and shoots of white spruce seedlings grown in Soil 2 and 3 and exposed to simulated acid rain for two and one half growing seasons

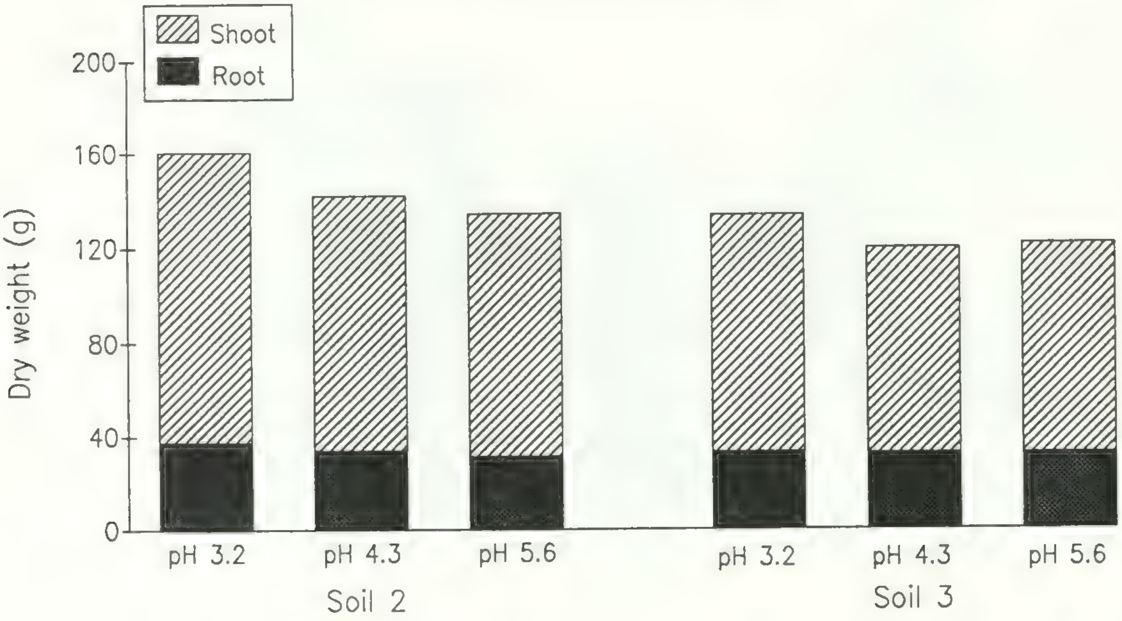
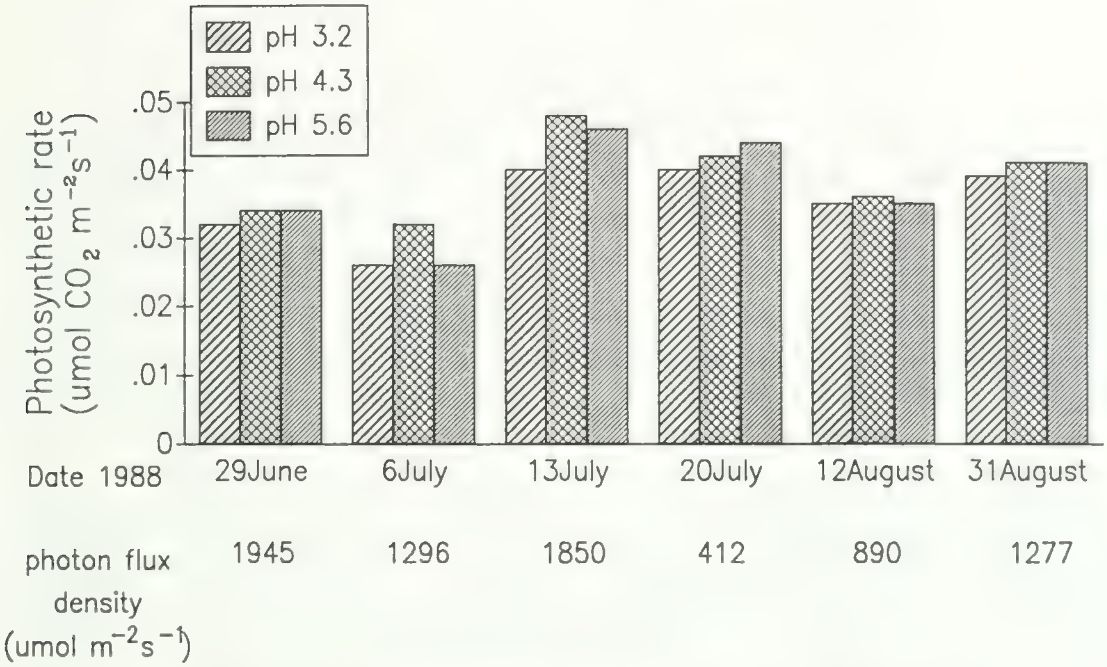


Figure 9: Photosynthetic rates of white spruce seedlings, taken on the dates indicated, which had been exposed to simulated acid rain during the growing season since June 1987



within any date, differences between columns are not significant ($p< 0.05$)

EFFECTS OF SIMULATED ACID RAIN ON THE GROWTH, NUTRITION AND PHYSIOLOGY OF SUGAR MAPLE SEEDLINGS GROWN WITHIN A MOBILE RAIN EXCLUSION CANOPY SYSTEM

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ABSTRACT

Simulated acid rain (SAR) with pH 3.2, 4.3 and 5.6 was applied to sugar maple seedlings (Acer saccharum Marsh.), aged 6 to 9 years, for two and one half growing seasons. Seedlings were transplanted from a natural forest site in Muskoka, Ontario, to pots of mineral soil. A mobile rain exclusion canopy (REC) system was used for treatment application and to exclude ambient rain. No significant treatment effects were observed on the height or stem diameter of the seedlings over the study period. Also, there were no significant effects on the shoot dry weight, leaf area, or leaf number measured at the end of the experiment. Analyses of 6 foliar pigments; chlorophyll a and b, B-carotene, antheraxanthin, violaxanthin and lutein failed to show any significant changes in pigment concentrations or alterations to the pigment ratios due to the SAR applications.

Foliar sulphur and nitrogen concentrations were significantly higher in the pH 3.2 treated seedlings compared to either the pH 4.3 or 5.6 treated seedlings. This was attributed to the higher sulphur and nitrogen inputs in the pH 3.2 SAR. The tendency toward greater growth in the pH 3.2 treated seedlings may be attributable to the higher sulphur and nitrogen inputs in this treatment. Foliar potassium concentrations indicated that the sugar maple seedlings were suffering from potassium deficiency but this was not attributable to the SAR treatments applied. There were no significant treatment effects on foliar calcium, magnesium, aluminum, iron or zinc concentrations. Overall, the results did not show that SAR had a detrimental effect on the sugar maple seedlings treated in this study.

INTRODUCTION

Sugar maple decline has been observed in parts of Ontario since 1947 (McIlveen et al. 1986). Major decline episodes which occurred in Ontario, Canada in the late 1950's were attributed to poor management (Griffin 1965) and in the late 1970's to forest tent caterpillar infestation (Gross 1980). Typically, the older studies attributed decline to climatic factors, poor management or disease organisms and did not consider effects of long range transport of air pollutants (Griffin personal communication). Recently the number of sugar maple decline episodes in Ontario has increased dramatically with reports of sugar maple declines from throughout the Great Lakes - St. Lawrence and the Deciduous Forest Regions (Linzon 1985). These regions encompass all of southern and central Ontario, as well as the southerly portions of northeastern and northwestern Ontario. In Quebec, severe and extensive sugar maple decline has been observed in the eastern townships area, south of Quebec city, since 1978 (Gagnon et al. 1985). The continued decline of this economically important species could jeopardize maple syrup production in these provinces.

Symptoms of decline have not changed since the earlier surveys. These include 1) sparse foliage on trees with leaves often dwarfed and exhibiting interveinal necrosis; 2) marginal leaf browning followed by premature leaf fall; 3) premature fall coloration; and 4) death of young twigs followed by dieback of part or all of the crown (Griffin 1965).

The hypothesis that air pollution has triggered a general forest decline or "Waldsterben" in Europe and the north east U.S.A. has fuelled speculation that air pollutants are responsible for recent declines of sugar maple in Canada (Houston, 1987). Skelly (1989) cautions that while declines of trees around point sources are well documented, biotic and abiotic factors can directly incite forest tree diseases without the need of air pollution as a predisposing factor. Nevertheless, there is increasing evidence that ozone and acid rain may be linked to the decline of sugar maple either directly through foliar injury and nutrient leaching or indirectly through Al toxicity due to increased soil acidification (Linzon 1985, McLaughlin et al. 1985, 1987).

A mobile rain exclusion canopy system (REC) was utilized to determine the impact of simulated acid rain (SAR) on the growth of sugar maple seedlings potted in mineral soil and placed in field plots from July 1987 to July 1989, in the presence of ambient ozone.

MATERIALS AND METHODS

This experiment was carried out in the REC system located at the Ontario Ministry of the Environment Phytotoxicology laboratory, Brampton Ontario. The operation of the REC system is explained in detail in a previous paper (Kuja et al. 1986).

Soil Requirements

In October 1985, 120 m^3 of soil was removed from a site on Margret Lake Rd. (Lot 1, Concession A, Ridout Twp.) This site is located south of Dorset, Ontario, approximately 1 km south of the Ministry of Natural Resources Leslie Frost Centre on Hwy. 35. Preliminary soil tests identified the soil as a humo-ferric podzol with a mineral B horizon low in base cations and having a pH of 5.7 (Table 1).

During soil collection, the organic layer (0 to 5 cm) was pushed aside and B horizon soil was removed to a depth of 50 cm using heavy machinery. The mineral soil was passed through a 2 cm screen in order to remove rocks and roots and to homogenize the material. The sieved soil was transported to the Phytotoxicology laboratory in Brampton and was used as a soil medium for native sugar maple transplants in the study.

Plant Culture

In October 1985, native sugar maple seedling transplants were removed from a site 1 km north of the site from which the study soil was excavated in Dorset, Ontario. Dendrochronological techniques determined that the seedlings were 6 to 9 years old when transplanted. Seedlings were selected on the basis of uniform height (35 to 40 cm) and similar branching pattern. At the collection site, the seedlings roots were washed in deionized water and packed in bags of moist sphagnum moss (25 per bag). The bare-root seedlings were transported to the Brampton study site and were kept in cold storage at 4°C for two weeks prior to transplanting into 16 litre, black, polyethylene pots containing the mineral soil described above. It was recognized that the seedlings would suffer from transplant shock for at least a year. In order to overcome this shock, the seedlings were kept in an outdoor establishment plot for 20 months prior to the start of the experiment. The transplants were sheltered by a 30 cm soil berm and partially shaded (40%) by a shade cover (Paraweb Inc.) which was erected 2 m above the tree seedlings.

Arrangement of Test Plants in REC Treatment Areas

Sugar maple transplants were exposed to SAR in three separate treatment areas. During ambient rain events, natural rainfall was excluded from these treatment areas by mobile greenhouse canopies. Each treatment area was divided into 25 plots (1.5m x 1.5m). Four potted sugar maple seedlings were transplanted to each plot within the treatment areas. SAR was applied from a nozzle centered 3 m above each plot when the canopies were parked over the treatment areas.

During the first week of June 1987, sugar maple seedling were graded in the establishment plot and only healthy individuals in soil with pH<6.0 were transplanted into the REC treatment areas. Each pot was buried to within 3 cm of the pot rim. Red pine bark chips were spread around all pots to prevent splashing of soil onto test plants during rain applications and to control weed growth.

Sugar maple is a climax species, the seedlings of which grow best under shaded conditions (Logan 1965). In order to provide shade and moderate the micro-environment in the treatment areas, hybrid poplar clones were planted in rows between treatment plots.
Rooted cuttings of fast-growing hybrid poplars clones (DN12, DN190 and OJPN105) were obtained from MNR Ontario Tree Improvement and Forest Biomass Institute (OTIFBI) in Maple, Ontario and planted into the REC system. However, these clones did not produce sufficient height or foliage to adequately shade the plots until late June, 1987. Therefore, black, PVC plastic, shade cover (Paraweb Inc.) was suspended on wires over the sugar maple-hybrid poplar plots to provide shade from late April to the end of June of 1987 at which time the hybrid poplars were large enough to provide shade to the sugar maples in the plots. The PVC covers were pulled off the treatment areas prior to each simulated acid rain applications. The PVC shade covers were not necessary in the subsequent years of the study. In 1988 and 1989, the hybrid poplars were regularly trimmed to 1.5 m in height to prevent interference with treatment application to the maple seedlings.

Treatment Chemistry and Application

Three SAR treatments were applied, pH 3.2, 4.3 and 5.6. The 5x5 latin square arrangement of nozzles dictated that two SAR treatments (i.e. pH 3.2 and pH 4.3 SAR) be assigned ten plots each, the third treatment (pH 5.6 SAR) was assigned five plots. The acidic pH 3.2 SAR treatment (650 ueq $H^+ L^{-1}$) was chosen to represent highly acidic rain events which occur at least once a year in Ontario. The pH 4.3 SAR treatment was selected (50 ueq $H^+ L^{-1}$) because this is the annual weighted mean pH of ambient rainfall in the southern Ontario region (Chan et. al. 1983). The pH 5.6 SAR treatment (12 ueq $H^+ L^{-1}$) was included as a control since this has been commonly accepted as the pH of unpolluted rain (Barrett and Brodin 1955).

SAR solutions were prepared from additions of acid/ion stock solutions to deionized water. Stock solutions, contained H_2SO_4/HNO_3 acids and mineral salts based on ambient rain chemistry in southern Ontario and had a final S:N mass ratio of 2:1 (Table 2). SAR was applied on Monday, Wednesday and Friday of each week in three 10 mm events (3.0 cm per week). Each event lasted 25 minutes.

During the month of June, 1987, deionized water was applied to plots to maintain soil moisture and allow tree seedlings and poplar cuttings to establish in the initial year of the study. SAR applications commenced on July 20 and were continued until October 23. In the second year, 1988, SAR was applied from May 10 to October 28. In the third and final year of the study, 1989, SAR was applied from May 15 to July 28.

Meteorological conditions, rain chemistry and ambient air concentrations of O_3, SO_2 and NO/NO_2 were monitored daily at the study site. Details of the pollution monitoring equipment are given in Dixon and Kuja (1990). Thermohygrographs were placed in stevenson screens centred in each treatment area and provided a continuous measurement of temperature and humidity at all times. Soil water potential was monitored in 1987 and 1989 with tensiometers distributed diagonally across the treatment area.

Measurements and Observations

Seedling height and stem diameter, at 5 cm above soil grade, were measured at least once a month during the growing season. Foliar injury (chlorosis and necrosis) were also assessed at these times. The number of leaves per plant was counted in May and July. Frequent counts during the first year indicated that the leaf number remained constant from June until the autumn leaf fall. In 1988 net photosynthesis rates (P.S.) (umol CO_2 m^{-2} s^{-1}) were determined weekly for one selected, unshaded maple leaf per plot using a Licor 6200 photosynthesis analyzer. The measured leaves were always selected from the first whorl of mature leaves on the west side of the seedling. These leaves were marked for subsequent measurements using coloured thread. P.S. measurements were always taken during early afternoon under uniform light conditions, generally clear skies.

The tallest seedling in each plot was removed in 1988 for detailed examination and was not included in the data set analyzed here. In total, data analysis was based on 225 seedlings (3 seedlings per plot x 25 plots per canopy x 3 canopy treatment areas).

Foliage was collected in August 1988 and analyzed for nitrogen, phosphorus, potassium, calcium, magnesium, sulphur, manganese, zinc, iron, and aluminum. The analytical methods used are given in Ontario Ministry of the Environment Handbook of Analytical Methods (1983). Chlorophyll, B-carotene, antheraxanthin, violaxanthin and lutein plant pigments were analyzed by extracting with methanol and chloroform and measurement by HPLC. The method is given in Appendix A.

Statistical Analyses

The slope of the plant height and stem diameter growth curves were calculated for the period from the beginning of May until the end of July for each of the years 1988 and 1989. The slopes were analyzed using a randomized complete block design. Analyses were carried out on a microcomputer using the statistics package SYSTAT (Systat Inc. 1988). Final height and diameter measurements were made at the end of July 1989 and were also analyzed as a randomized complete block design with the initial height or diameter (June 1987) used as the covariate. Foliar elemental analysis, pigment analysis and shoot weight were analyzed as randomized complete block designs. Photosynthesis was analyzed as a repeated measures design.

RESULTS

Rain Events and Air Quality.

In 1987, the initial year of the study, a total of 247 mm of ambient rain fell in 45 events during the treatment application period (July 20 to October 23) period. During this time, a total of 400 mm of SAR was applied in 40 predetermined events. In the second year of the study, SAR treatments commenced on May 10 and continued until October 28, 1988. A total of 688 mm of SAR was applied in 70 events compared with 397 mm of ambient rainfall which occurred over 80 events during the same period. In the third and final year, treatments were applied from May 15 to July 31. During this 11 week period, 340 mm of SAR was applied in 33 events. A total of 227 mm of ambient rainfall occurred in this time in 27 events. No ambient rainfall occurred between June 24 to July 20, 1989.

The seasonal 12 hour mean ozone concentration during 1987 was 0.020 ppm. The seasonal means for SO_2, NO_2 and NO were 0.002, 0.004 and 0.009 ppm respectively. The highest ambient ozone concentration recorded during the growing season was 0.101 ppm on August 15, 1987. Ambient ozone levels were higher in 1988 than in 1987. The 1988 seasonal 12 hour mean ozone concentration was 0.029 ppm and the 1988 seasonal peak was 0.156 ppm which occurred on July 6. In 1989, the maximum mean half hour concentration of ozone was 0.091 ppm which occurred on July 23.

The pattern of growth in height of sugar maple seedlings from June 1987 to the end of July 1989 is shown in Figure 3. On average, seedlings increased in height by 40 cm. Approximately 88% of the total growth over the two and one half year period occurred in May and June 1989. There was no significant treatment effect in any year, however, by the end of the experiment, the pH 3.2 and 4.3 treated plants tended to have grown more than the pH 5.6 sprayed seedlings.

The increase in stem diameter from 1987 through to the end of July 1989 can be seen in Figure 4. There was little measurable radial stem growth in 1987, but a steady increase in growth in both 1988 and 1989. There was neither a significant difference among the slopes of the stem diameter growth curves over the three years, nor was there a significant difference in the stem diameter increase amongst treatments at the end of the experiment.

Figures 5 to 7 show the mean shoot weight, leaf area and number of leaves of the sugar maple seedlings at the end of the two and one half year study period. There were no significant treatment effects in any of these parameters. The pH 5.6 treatment produced the lowest values for each parameter, as was the case for stem height and diameter.

Foliar concentrations of chlorophyll, lutein, B-carotene, violaxanthin and antheraxanthin plus pigment ratios are given in Table 3. There were no significant differences in either the pigment concentrations or the ratios due to the treatments applied.

Table 4 shows the foliar concentrations of N, P, K, S, Ca, Mg, Mn, Zn, Fe and Al in sampled sugar maple seedlings. Foliar samples were collected in late summer 1988. The concentrations of nitrogen, sulphur and manganese were significantly higher in the foliage of the pH 3.2 treated seedlings than the foliage from the other two treatments. In 1988 there was no evidence of a reduction in calcium or magnesium in the pH 3.2 treatment relative to the pH 4.3 or 5.6 SAR treatments.

DISCUSSION

This study was conducted to determine whether acid precipitation is having an effect on the growth, nutrition and phyisology of sugar maples in Ontario. The major declines documented in Quebec throughout the 1980's have not occurred in Ontario although McLaughlin (1990) documented an increase in sugar maple decline in 1987. Although several factors, including insect defoliation and drought, account for many of the symptoms of the decline, they do not account for all the symptoms (Bernier et al. 1989). Acidic deposition may be a significant predisposing stress on maple and a significant link in the decline cycle. The results of the REC study indicate that SAR of pH 4.3 and 3.2 had no significant effect on stem height or diameter growth, shoot dry weight, leaf area, photosynthetic rate, chlorophyll concentration or nutrient status of the sugar maple seedlings tested.

The SAR applications totalled more than the ambient rainfall. This was in part because the summers of 1987, 1988 and 1989 were abnormally hot and dry. Also the experiment was designed to ensure the seedlings had sufficient water and were not suffering from drought stress. It is, however, recognized that the interaction between acid rain stress and drought stress may be important in forest decline.

Ozone, sulphur dioxide and nitrogen oxides were not excluded from the treatment areas and may have affected the growth of the seedlings. Ambient sulphur dioxide and nitrogen oxide concentrations were not sufficiently elevated to cause direct damage. Ozone concentrations were frequently high in both 1987 and 1988 due to dry sunny weather conditions. In spite of this, no symptoms of ozone injury were seen on the maple seedling foliage even after an event of several hours duration on July 6, 1988 which peaked at 156 ppb ozone. Kress and Skelly (1982) report that sugar maple seedlings exposed to 150 ppb ozone, 6 hours per day for 28 days had only 60% of the total dry weight of control plants grown in filtered air. Seedlings grown in 100 ppb ozone for the same treatment period had much less injury, having 93% of the total dry weight of control plants grown in filtered air.

Mean monthly concentrations of ozone in the REC system were below 50 ppb throughout the experiment and even in 1988, the year with highest ozone concentrations, the mean ozone concentration was above 100 ppb for only 24 hours. Therefore, it is unlikely that ambient air pollution had a major impact on these REC sugar maple seedlings or that the ambient pollution concentrations significantly altered the experimental results.

The theory that acidic precipitation may acidify forest soils to the point at which aluminum is mobilized has received a great deal of attention over the past decade. Aluminum toxicity has been widely recognized as causing root damage in agricultural crops and trees. Generally Al^{3+} reaches phytotoxic concentrations when the soil is acidified to within the aluminum buffering range of pH 4.2 to 2.8. The soils used in the Ministry of the Environment experiment had a pH within the silicate buffering range of pH 5.0 to 6.2 which suggests aluminum toxicity should not be a problem. However, Ulrich (1983) has pointed out that the pH recorded around European beech roots, at depths up to 60 cm, were below pH 4.2 even though the soils were developed on limestone. He attributes this acidification of calcareous soils to large volumes of acidic stemflow funnelling down the beech trunks and penetrating into the soil along the roots. This suggests that a similar acidification may be possible, with the consequent aluminum mobilization, in the soil used in the REC experiment. The beech trees referred to by Ulrich (1983) were 110 years old and had experienced acidic deposition for decades. The sugar maple seedlings in the REC experiment funnelled very small quantities of water to the stem bases, the leaves tended to shed the spray solutions away from the stem bases and no change in soil pH was measured during the two and one half years over which the experiment was conducted. Clearly the determination of major alterations in soil chemistry requires a much longer experimental period than 2.5 years.

Average shoot weight, leaf weight, leaf number and leaf area all reflect the general pattern of growth observed in the height and stem diameter measurements (Figure 5 to 7). Although there were no significant treatment effects, the pH 5.6 treated seedlings tended to be smaller than the seedlings exposed to the other two treatments. The tendency toward better growth in the plants exposed to pH 3.2 and 4.3 SAR suggests these treatments, with their higher sulphur and nitrogen inputs, may have had a slight fertilizer effect.

Photosynthetic measurements made throughout the growing season in 1988 showed no significant treatment effects. Photosynthetic rates were consistent among the measurement dates. This finding supports the findings of Jurik (1986) who found the photosynthetic rates of sugar maples were constant from the time of full leaf expansion to senescence, assuming other variables such as light levels are held constant. This implies that measurements of the stress due the treatments applied could be measured at any time within this period, and time of measurement could be eliminated as a variable. This does not eliminate the possibility that the SAR treatments could have a cumulative effect. However, a consistent pattern of photosynthetic rate compared to the treatment applied was observed, indicating that there is no pattern of alteration in photosynthetic rate which could be attributed to the SAR treatments (Figure 8).

Photosynthetic measurements were taken on the same set of leaves throughout the growing season (Figure 8). It is recognized that one leaf from one seedling per plot does not provide a good estimate of the photosynthetic rate of all the seedlings receiving any one treatment. Nevertheless, it is noteworthy that the pH 4.3 treated seedlings had consistently higher photosynthetic rates than the seedlings in the other treatments. This tendency toward greater photosynthesis in the pH 4.3 treated seedlings is also reflected in shoot dry weight and leaf area (Figure 5 and 6).

Forest decline often increases during periods of drought (Schulze 1989). The 1988 increase in the decline index of sugar maple trees in Ontario followed a hot dry summer in 1987 and 1988 (McLaughlin 1990). Ulrich and Matzner (1988) point out that anthropogenic acidification is reinforced by natural acidification in warm, dry years. Exacerbation of acid rain damage by drought could not be detected in the REC experiment since the maple seedlings were regularly watered with SAR. Soil tensiometers located throughout the treatment plots had mean readings of 20.8 ($^{+}/_{-}$10.2 kPa indicating the soils were well supplied with water.

Pigment concentrations were not significantly different among treatments (Table 3), nor were the pigment ratios altered by the treatments. Sheridan and Rosenstreter (1980) studying the effect of acidity on chlorophyll in the moss *Tortula ruralis*, found that chlorophyll *A* was more readily degraded by high hydrogen ion concentrations than chlorophyll *B*, ie. the lower the ratio of chlorophyll *A* to *B*,, the greater the damage. In the REC study, there was no indication of alteration in the chlorophyll ratios.

Wolfenden *et al.* (1988) while studying the early diagnosis of forest decline, found ethylene emissions were much higher in needles from declining trees compared to ethylene emissions from healthy trees. They also found a good correlation (r= 0.873) between ethylene emissions and the ratio of violaxanthin to antheraxanthin. They suggested that an increase in the violaxanthin to antheraxanthin ratio is an indication of tree decline. The ratios in Norway spruce needles they analyzed were between 1.0 and 1.7. In the REC system the violaxanthin to antheraxanthin ratios were from 0.95 to 1.0 in the maple leaves, with a trend toward a lower ratio with increasing treatment acidity. This suggests that the leaves receiving the pH 3.2 treatment are healthier than the leaves receiving either the pH 4.3 or the pH 5.6 treatments.

Foliar nutrient concentrations of nitrogen, sulphur and manganese were higher in the pH 3.2 treatment than in the other treatments (Table 4). Nitrogen concentrations were below the critical level for sugar maples, as given by the Ontario Ministry of Natural Resources Research Report (1972), in both the pH 4.3 and 5.6 treatments yet above the critical level in the pH 3.2 treated seedlings. This suggests that the acid rain solutions had a slight fertilizer effect which, in the nutrient poor mineral soil used, may be important to the seedlings growth. However, growth of the pH 3.2 treated seedlings tended to be less than the pH 4.3 treated seedlings, which may reflect a loss of other nutrients, such as potassium, due to leaching by the most acidic SAR treatment.

Potassium concentrations in the foliage of sugar maple seedlings in all treatments were extremely low, approximately 4300 ppm, reflecting low soil concentrations (Table 4). Foliar potassium concentrations in mature sugar maples, even on declining sites in Ontario, are generally over 7000 ppm (McLaughlin 1990). The deficiency of potassium undoubtedly affected the growth of the seedlings, although in 1989 most seedlings experienced a strong growth increase. Bernier et al. (1989) report that sites in Quebec with foliar potassium concentrations below 5500 ppm exhibited high levels of decline. Since the REC seedlings were already stressed with potassium deficiency, the increased foliar leaching with acid rain could exacerbate the problem. Kuja and Dixon (1988) report elevated potassium concentrations in pH 3.2 rain droplets on maple foliage compared to pH 5.6 droplets. There was, however, no indication that the acid rain treatments applied altered the foliar potassium concentrations of sugar maple seedlings in the REC system in either 1987 or 1988.

Bernier and Brazeau (1988) observed visual symptoms of phosphorus deficiency in upper crown foliage of sugar maple trees in Quebec with phosphorus concentrations of 1110 ppm. Other studies they referenced reported much higher concentrations, ranging up to 2700 ppm. Our foliar concentrations of approximately 2600 ppm are within this normal range of foliar concentrations. The visual symptoms of phosphorus deficiency (ie. small purple leaves) were not evident in the REC sugar maple seedlings. Pare and Bernier (1989) report that sugar maple stands with inadequate phosphorus nutrition were those with mull humus compared to mor humus. They state that this may occur because the P cycling in a mor humus is confined to the organic horizons and not the mineral horizons, whereas in a mull humus soil, there is a great deal more mixing between the mineral soil and organic horizons. Acidification of the soil will lead to a increased fixation of phosphorus by iron and aluminum oxides.

The data in Table 4 suggests that reduced availability may have occurred in the REC soil since the foliar P concentrations were lowest in the pH 3.2 treatment compared with either the pH 5.6 or 4.3. The point at which decreased availability of phosphorus may be critical is in times of drought. Westing (1966) points out that arboriculturists fertilize with phosphorus to protect sugar maple trees from drought damage. Although the phosphorus concentrations appear to be adequate, reductions in phosphorus availability due to acidification coupled with drought stress could lead to the decline of the trees.

European studies have identified magnesium deficiencies as a major problem associated with forest decline. Similarly in Quebec, Bernier and Brazeau (1988) report areas where magnesium deficiency may be linked to maple decline. The low concentrations of magnesium are generally attributable to magnesium poor soils and decreases in exchangeable magnesium may be attributed to leaching by acid deposition (Johnson 1989). Although, with time, the leaching loss of magnesium from soils with good supplies of magnesium may be significant, concern to date is only on magnesium poor soils. The soil used in the REC experiment was relatively high in both magnesium and calcium and therefore, deficiency of either of these nutrients, even over a longer period of acidification, is unlikely.

The most acidic treatment applied in the REC experiment, pH 3.2, was an order of magnitude more acidic than the average pH of ambient rainfall in southern Ontario and yet the seedlings tested showed no adverse reactions to this treatment. This indicates that these sugar maple seedlings are not sensitive to acid rain. Schutt and Cowling (1985), in describing forest decline in Europe, note that younger trees are generally less affected than older trees.

In Ontario the concern that sugar maple trees were experiencing a decline which could be as severe as in Quebec has not materialized. McLaughlin (1990) reports that sugar maple stands in Ontario monitored for decline since 1984 showed an increase in decline following forest tent caterpillar defoliation and hot dry summers in 1987 and 1988. However, in 1989 the decline index had decreased back to 1984 levels. Comparisons of genetic differences between mature sugar maples and sugar maple seedlings in Ontario have not been conducted.

Knowles and Perry (1990) investigated the genetic differences between mature sugar maples which are declining and those which are healthy. Overall, they did not find an increase in heterozygosity of healthy mature sugar maple trees compared to declining trees, nor was there evidence that alleles present in sensitive subpopulations might be endangered due to the death of sensitive trees. Therefore, selection for pollution tolerance is not occurring at the level of mature trees, even if one assumes that air pollution has at least some effect on the health of mature trees.

The results of the MOE study suggest that selection for acid rain tolerance is not taking place at the seedling stage. This suggests that acid rain is not altering the genetic makeup of the sugar maple population in Ontario.

CONCLUSION

Sugar maple seedlings transplanted from a natural stand into mineral soil obtained from an area impacted by acid rain and having generally sensitive soils showed no significant growth effects due to simulated acid rain treatments with a pH as low as 3.2. The pH 3.2 and 4.3 treated seedlings tended to have greater growth and photosynthetic rate than seedlings treated with pH 5.6 rain. This suggests that the nitrogen and sulphur inputs in the SAR solutions may have a fertilizer effect on these plants. This hypothesis is supported by significantly higher nitrogen and sulphur concentrations in the foliage of the pH 3.2 treated seedlings. Pigment analysis of the foliage failed to show any alteration in pigment concentrations which could be attributed to acid rain stress or alterations in pigment ratios which might serve as an early diagnosis of decline.

This experiment was too short in duration to study the effects of acidification of the soil, long term cation leaching or aluminum mobilization on the growth of sugar maple seedlings. The results of this study indicate that sugar maple seedlings are not sensitive to simulated acid rain even ten times as acidic as occurs on average in Ontario. The effect of long term alterations in soil chemistry attributable to acid rain could not be assessed in the two and one half years over which the study was conducted. The alteration of soil chemistry is a critically important factor for the long term survival and growth of this species in Ontario.

REFERENCES

Barrett, E. and G. Brodin. 1955. The acidity of scandinavian precipitation. Tellus 7: 251.

Bernier, B. and M. Brazeau, 1988. Magnesium deficiency symptoms associated with sugar maple dieback in a Lower Laurentians site in southwestern Quebec. Can. J. For. Res. 18: 1265.

Bernier, B., D. Paré and M. Brazeau. 1989. Natural stresses, nutrient imbalances and forest decline in southeastern Quebec. Water, Air and Soil Pollut. 48: 239.

Chan, W.H., A.J.S. Tang and M.A. Lusis, 1983. Precipitation concentration and wet deposition fields of pollutants in Ontario September 1980 to December 1981. Report No. ARB-61-83-ARSP. Ontario Ministry of the Environment.

Gagnon, G., L. Robitaille, G. Roy, C. Gravel. 1985. Dieback in maple stands - the behaviour of some ecological variables. Presented at the NATO Advanced Research Workshop on Effects of Acid Deposition and Air Pollutants in Forests, Wetlands and Agricultural Ecosystems. Toronto, Canada. pp 1-17.

Griffin, H.D. 1965. Maple dieback in Ontario. For. Chron. 41: 295.

Gross, H.L. 1980. Maple decline in Ontario: decline status 1979 compared to 1978 including starch food reserve analysis. Forest Insect and Disease Survey- GLFRC: 1-6.

Houston, D., 1987. Forest tree declines of past and present: current understanding. Can. J. Plant Path. 9: 349.

Jurik, T.W. 1986. Seasonal patterns of leaf photosynthetic capacity in successional northern hardwood tree species. Amer. J. Bot. 73: 131.

Kress, L.W. and J.M. Skelly. 1982. Response of several eastern forest tree species to chronic doses of ozone and nitrogen dioxide. Plant Disease 66: 1149.

Knowles, P. and D.J. Perry. 1990. Genetic characteristics of healthy and declining sugar maple (Acer saccharum Marsh.) in Ontario. Ontario Ministry of the Environment project number 435G.

Kuja, A.l., R. Jones, A. Enyedi, 1986. A mobile rain exclusion canopy system to determine dose-response relationships for crops and forest species. Water, Air, Soil Pollut. 31: 417.

Kuja, A.L. and M.J. Dixon. 1987. Five year study using a mobile rain exclusion canopy system to determine joint effects of simulated acid rain and ozone on the growth and physiology of sugar maple and white spruce seedlings. In: Proceedings of the Technical Transfer Conference, Part A, Air Quality Research, Royal York Hotel, Toronto, Nov. 30 to Dec. 1, 1987.

Linzon, S.N., 1985. Forest damage and acidic precipitation. In: E.B. Eddy Distinguished Lecture Series Nov. 1985, Faculty of Forestry, Univ. of Toronto, Toronto, Ontario.

Logan, K.T. 1965. Growth of tree seedlings as affected by light intensity. I. white birch, yellow birch, sugar maple and silver maple. Can. Depart. of For. Publ. 1121

McIlveen, W.D. and S. Rutherford, 1986. A Historical Perspective of Sugar Maple Decline within Ontario and Outside of Ontario. Report No. ARB-141-86-PHYTO. Ontario Ministry of the Environment.

McLaughlin, D.L., 1987. Maple decline in Ontario: Situation research status report. M.O.E. Report prepared from Maplefest 87, Grand Falls, N.B. June 12-13, 1987. 30p.

McLaughlin, D.L. 1990. Etiology of sugar maple decline at selected sites in Ontario (1984-1989). Report No. ARB-293-89-PHYTO. Ontario Ministry of the Environment.

McLaughlin, D.L., S.N. Linzon, D.E. Dimma, W.D. McIlveen, 1985. Sugar maple decline in Ontario. Report No. ARB-144-85-PHYTO. Ontario Ministry of the Environment.

McLaughlin, D.L., D.E. Corrigan, W.D. McIlveen, 1987. Etiology of Sugar Maple Decline at Selected Sites in Ontario (1984-1987). Report ARB-202-87-PHYTO. Ontario Ministry of the Environment.

NAPAP 1989. Annual Report, 1989 and Findings Update. National Acid Precipitation Assessment Program, Washington, D.C.

Ontario Ministry of the Environment 1983. Handbook of analytical methods for environmental samples. Vol. 1 and 2. Laboratory Services Branch of the Ontario Ministry of the Environment.

Ontario Ministry of Natural Resources 1972. Annual Program Report of the Forest Research Branch.

Paré, D. and B. Bernier. 1989. Origin of the phosphorus deficiency observed in declining sugar maple stands in the Quebec Appalachians. Can. J. For. Res. 19: 24.

Schulze, E.-D. 1989. Air pollution and forest decline in a spruce (Picea abies) forest. Science 244: 776.

Schutt, P. and E.B. Cowling. 1985. Waldsterben, a general decline of forests in central Europe: symptoms, development, and possible causes. Plant Disease 69: 548.

Sheridan, R.P. and R. Rosenstreter. 1973. The effect of hydrogen ion concentration in simulated rain on the moss Tortula ruralis (Hedw.) Sm. Bryologist 76: 168.

Skelly, J.M. 1989. Forest decline versus tree decline- the pathological considerations. Environ. Monitor. Assess. 12: 23.

Ulrich, B., 1983. Soil acidity and its relations to acid deposition. IN: B. Ulrich and J. Pankrath (eds.) Effects of Air Pollution on Forest Ecosystems. Reidel Publ., Dordrecht, F.R.G. pp. 1-32.

Ulrich, B. and E. Matzner. 1988. Research Sites in the Solling Area. IN: B. Krahl-Urban, H.E. Papke, K. Peters and Chr. Schimansky. Forest Decline Assessment Group for Biology, Ecology, and Energy of the Julich Nuclear Research Center. Julich, F.R.G.

Westing, A.H. 1966. Sugar maple decline: an evolution. Econ. Bot. 20: 196.

Wolfenden, J, D.C. Robinson, J.N. Cape, I.S. Paterson, B.J. Francis, H. Mehlhorn and A.R. Wellburn. 1988. Use of carotenoid ratios, ethylene emissions and buffer capacities for the early diagnosis of forest decline. New Phytol. 109: 85.

Table 1. Texture and Chemistry of the mineral soil in which the REC study sugar maple seedlings were grown.

Soil Parameter			
pH (H_2O)	5.6-6.0	K (mg.g^{-1})	4.8
pH ($CaCl_2$)	5.0-5.5	Al (mg.g^{-1})	0.014
% Sand	90	N (mg.g^{-1})	14.9
Silt	7	P (mg.g^{-1})	0.91
Clay	3	Fe (mg.g^{-1})	0.06
% Organic C	2.4	S (mg.g^{-1})	1.1
Ca*(cmol(+).kg^{-1}	7.96	Cu (mg.g^{-1})	16
Mg*(cmol(+).kg^{-1}	0.36	Ni (mg.g^{-1})	2
K *(cmol(+).kg^{-1}	0.03	Pb (mg.g^{-1})	5
Al*(cmol(+).kg^{-1}	0.20	Cd (mg.g^{-1})	0.2
Ca (mg.g^{-1})	18.4	Zn (mg.g^{-1})	20
Mg (mg.g^{-1})	3.1		

Table 2. Chemical composition of simulated acidic rain (SAR) solutions.

SAR• pH	H^+ ueq L^{-1}	Acid vol. ml L^{-1} H_2SO_4	HNO_3
5.6	12	0	0
4.3	50	$1.001\ E^{-3}$	$8.849\ E^{-3}$
3.2	650	$1.301\ E^{-2}$	$1.151\ E^{-2}$

• All simulated rain solutions contained the following background ions (mg L^{-1}):

K - 0.063

Mg - 0.103

Na - 0.100

Cl - 0.023

Ca - 0.501

NH_4 - 0.744

NO^3 - 1.385

SO^4 - 2.771

S:N ratio = 2:1

Table 3: Pigment Concentration of Sugar Maple Seedlings Treated for Two Growing Seasons with Simulated Acid Rain

PIGMENT	pH 3.2	pH 4.3	pH 5.6
Violaxanthin	106	144	143
Antheraxanthin	111	148	143
Lutein	356	399	406
Chlorophyll a	2834	3031	2811
Chlorophyll b	1149	1228	1316
B-carotene	99	118	98
RATIOS*			
chlora/b	2.5	2.5	2.3
caro/chlora	.04	.04	.06
caro/xanth	.17	.19	.15
chlor/car	6.00	5.66	5.44
viol/lutein	.30	.32	.33
viol/anth	.95	.97	1.00

* Ratio abbreviations

chlora= chlorophyll a
chlorb= chlorophyll b
caro = B-carotene

xanth = antheraxanthin+ violaxanthin+lutein
anth = antheraxanthin
viol = violaxanthin

Table 4. Elemental analysis of leaves of sugar maple seedlings grown in mineral soil and exposed to simulated acid rain (ug/g dry weight).

Element	pH 3.2	pH 4.3	pH 5.6	Critical Level*
N	16000(a)	15000(b)	15000(b)	15620
P	2500	2700	2600	1220
K	4200	4300	4300	4680
S	1300(a)	1000(b)	1000(b)	
Ca	20000	19500	20000	17340
Mg	2800	2800	2700	1520
Mn	83(a)	65(b)	67(b)	
Zn	10	10	11	
Fe	150	170	150	
Al	100	130	100	

* critical levels are from the MNR Forest Research Annual Report 1972.

Foliar elemental concentration values followed by the same letter, or no letters, are not significantly different ($p<0.05$) among treatments.

Figure 1: Diagram of the Rain Exclusion Canopy System treatment plots

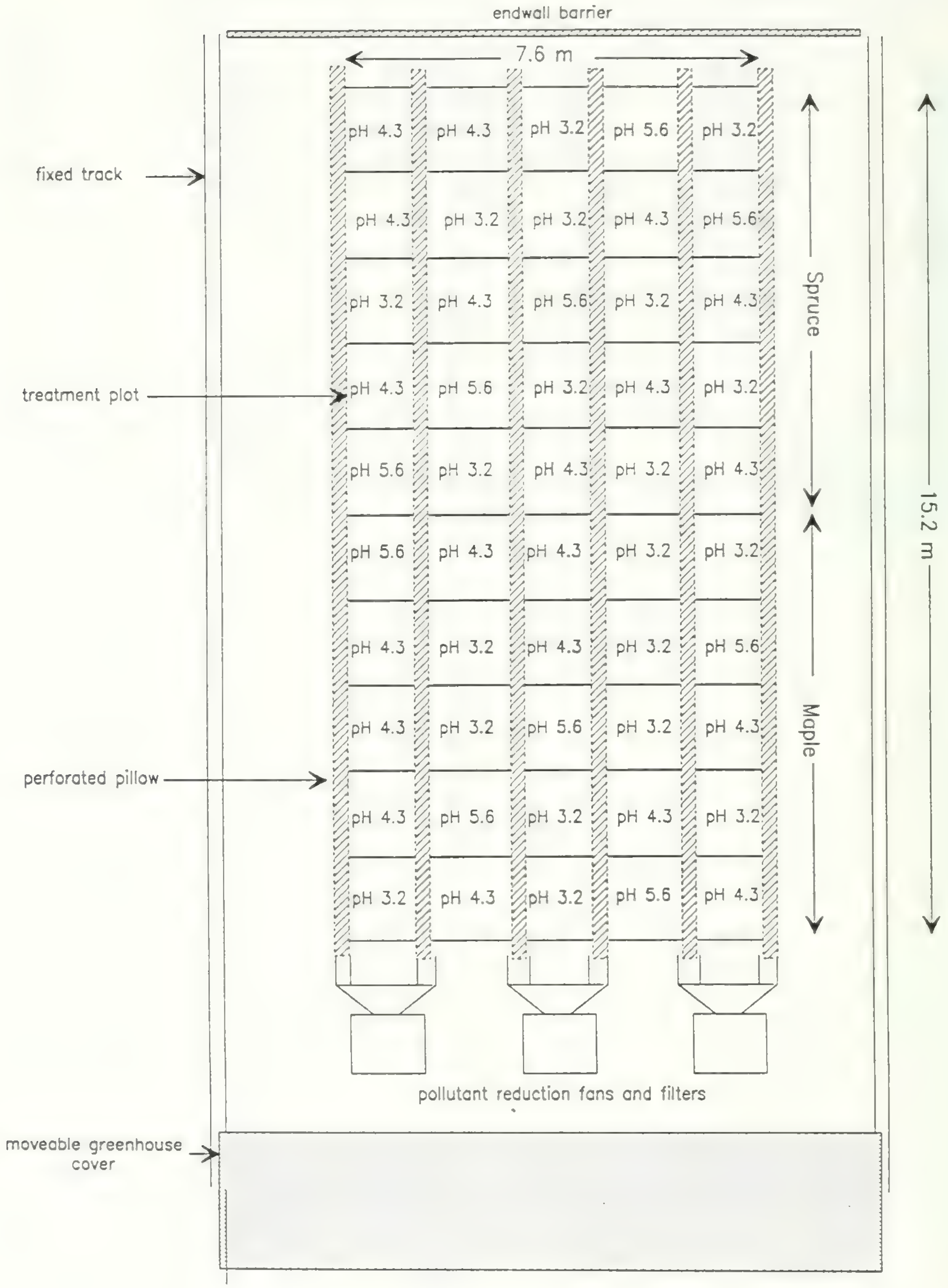

endwall barrier
7.6 m
pH 4.3 pH 4.3 pH 3.2 pH 5.6 pH 3.2
pH 4.3 pH 3.2 pH 3.2 pH 4.3 pH 5.6
pH 3.2 pH 4.3 pH 5.6 pH 3.2 pH 4.3
pH 4.3 pH 5.6 pH 3.2 pH 4.3 pH 3.2
pH 5.6 pH 3.2 pH 4.3 pH 3.2 pH 4.3
pH 5.6 pH 4.3 pH 4.3 pH 3.2 pH 3.2
pH 4.3 pH 3.2 pH 4.3 pH 3.2 pH 5.6
pH 4.3 pH 3.2 pH 5.6 pH 3.2 pH 4.3
pH 4.3 pH 5.6 pH 3.2 pH 4.3 pH 3.2
pH 3.2 pH 4.3 pH 3.2 pH 5.6 pH 4.3
Spruce
Maple
15.2 m
fixed track
treatment plot
perforated pillow
pollutant reduction fans and filters
moveable greenhouse cover

Figure 2: Placement of Sugar Maples and Hybrid Poplars in Rain Exclusion Canopy Plots

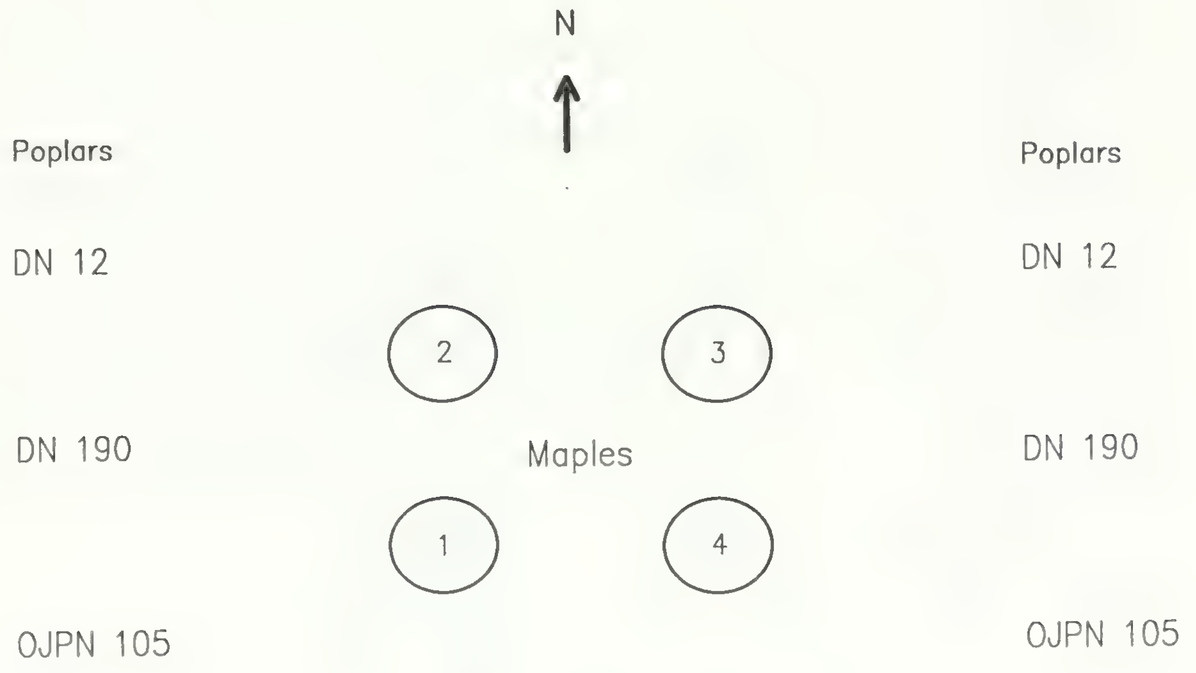


Figure 3: Height increase of sugar maple seedlings exposed to simulated acid rain for two and one half growing seasons

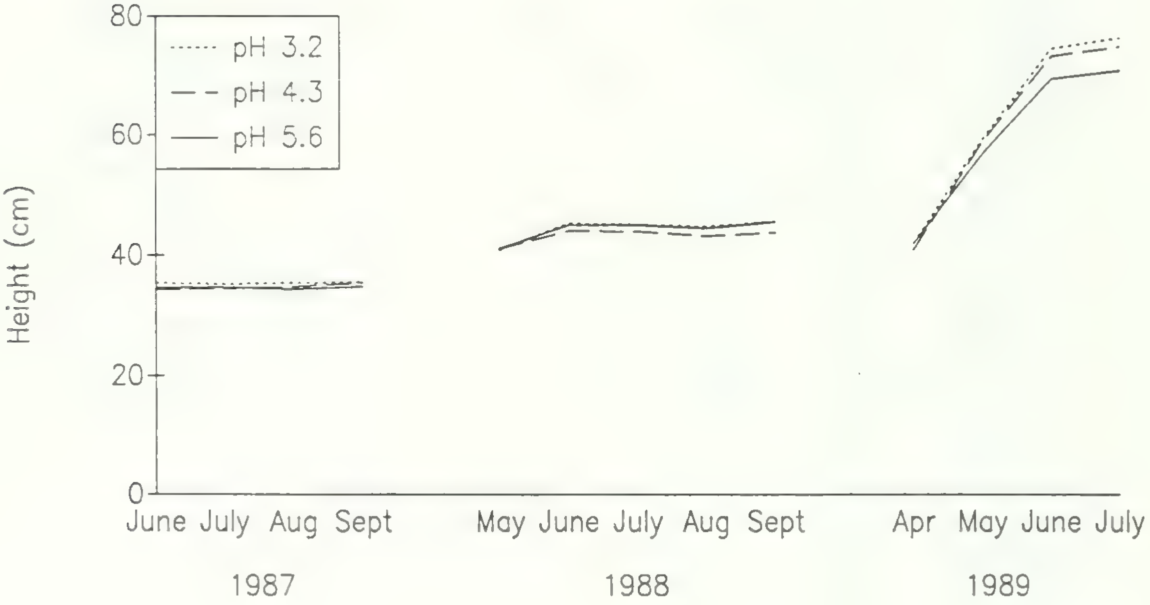


Figure 4: Stem diameter of sugar maple seedlings exposed to simulated acid rain for two and one half growing seasons

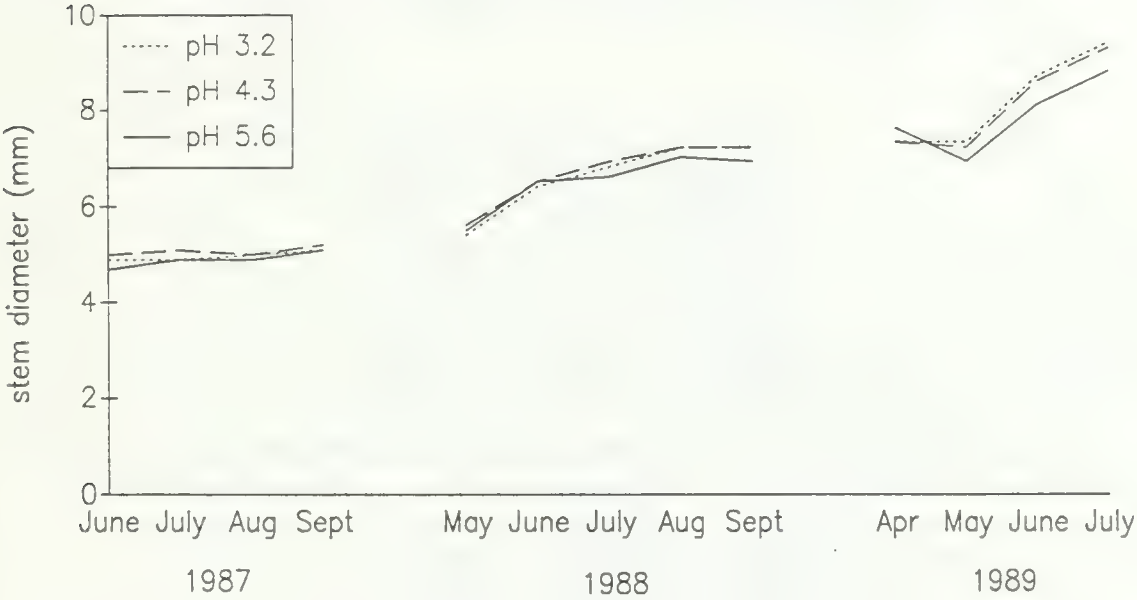


Figure 5: Shoot weight of sugar maple seedlings exposed to simulated acid rain for two and one half growing seasons

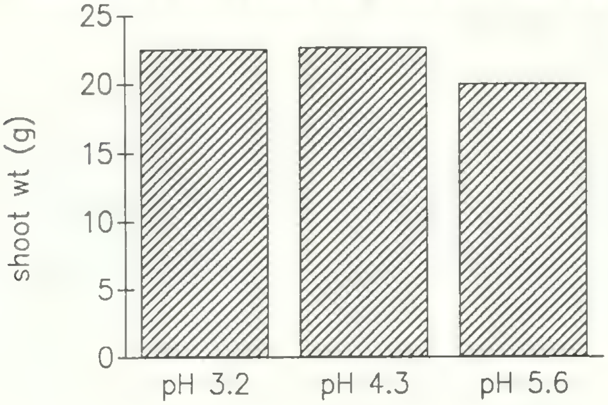


Figure 6: Leaf area of sugar maple seedlings exposed to simulated acid rain for two and one half growing seasons

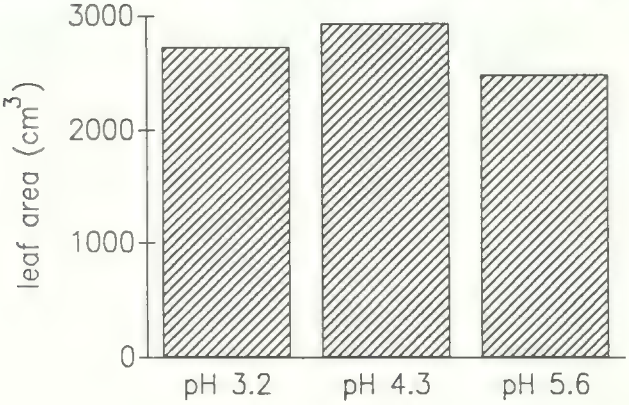


Figure 7: Leaf number of sugar maple seedlings exposed to simulated acid rain for two and one half growing seasons

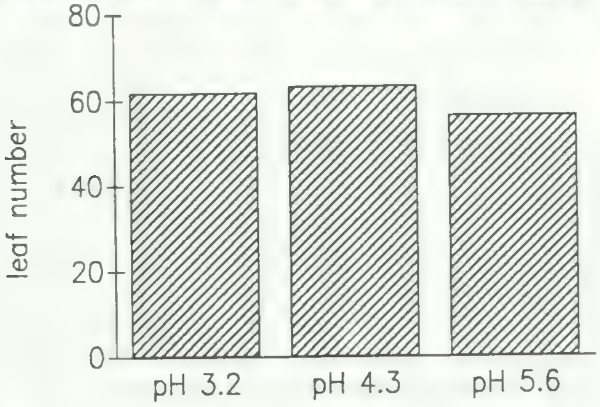
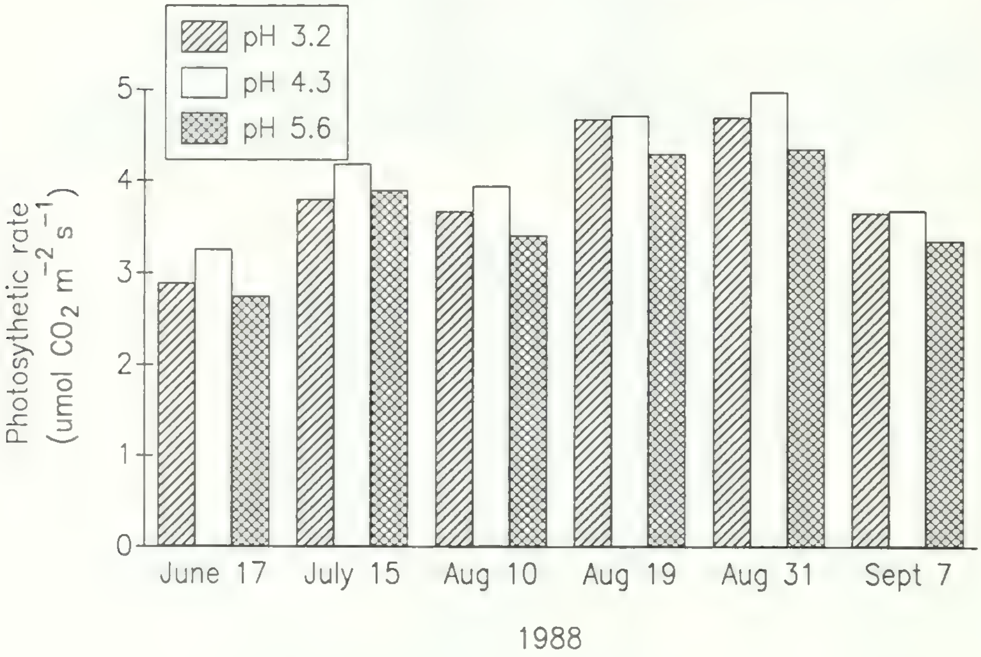


Figure 8: Photosynthetic rate of sugar maple seedlings in the second year of simulated acid rain applications



THE EFFECT OF SIMULATED ACID RAIN ON THE GROWTH OF SUGAR MAPLE SEEDLINGS

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INTRODUCTION

Sugar maple seedlings commonly establish on the organic surface soil layers when in natural stands. However, extensive areas of mineral soil are exposed when an area is logged. These areas will form the seedbed for the sugar maple seedlings. This experiment was designed to study the effect of simulated acid rain (SAR) on seedling establishment. It was conducted as part of a larger study to determine the effects of simulated acid rain on the growth, nutrition and physiology of sugar maple seedlings which were transplanted from a natural forest site in Muskoka, Ontario to pots of mineral soil (Kuja and Dixon, 1991). The study was conducted within a mobile rain exclusion canopy system located at the MOE Phytotoxicology controlled environment laboratory, Brampton, Ontario.

MATERIALS AND METHODS

Sugar maple seeds were obtained from a single road-side tree situated east of Bronte Creek in Halton County in southern Ontario, near the town of Palermo (Hwy 5 and 25). Sugar maple seeds were not available for experimental purposes from the Muskoka region (in the vicinity of Dorset) because the seed crop was very poor during the mid 1980s. The seeds were stratified at 4°C for 3.5 months in moist sphagnum moss. The seeds broke dormancy by mid February, 1988, and were transferred to 12.5 cm plastic pots containing soil collected from a natural maple stand located near Dorset, Ontario. This area experienced sugar maple decline in the mid 1980s and is an area which receives high loadings of acid precipitation and has sensitive soils. The soil consisted of B horizon material of a humo-ferric podzol with a pH ($CaCl_2$) of 4.5. The chemical composition of the soil, collected near the Ontario Ministry of the Environment research site at Plastic lake, is given in Table 1.

The seedlings were grown under controlled conditions in a greenhouse until the 14 June 1988. At this time, 75 were transplanted into 16 L pots also containing Plastic lake soil and moved into the rain exclusion canopy (REC) system used to exclude ambient rain and apply simulated acid rain (SAR). This system is described in detail by Kuja et. al. (1986). The position of the seedlings within the canopy system is given in Figure 1.

Simulated acid rain treatments were prepared by the addition of H_2SO_4/HNO_3 acids and mineral salts to deionized water (Table 2). The mineral salts were added in concentrations which approximated the average ambient rain chemistry in southern Ontario. Simulated acid rain (SAR) of pH 3.2, 4.3 or 5.6 was applied on Monday, Wednesday and Friday of each week, from June to October, 1988 and May to August, 1989. Ten mm of SAR was applied in each event. In total 30 seedlings received pH 3.2 SAR, 30 seedlings received pH 4.3 SAR, and 15 seedlings received pH 3.2 SAR.

Stem height, measured from the soil level, and stem diameter, measured 5 cm above the soil, were recorded at least once a month from June to October in 1988 and from May to August 1989. The seedlings were harvested on August 9, 1989. For each seedling, the leaf number was counted and the leaf area measured on a LI-3100 area meter, LI-COR corp. Lincoln Nebraska, U.S.A. The leaves and stems were then dried at 60°C for 24 hours and weighed. The root system of each plant was carefully washed from the soil. The root area was measure on the LI-3100 area meter and then dried at 60°C and weighed.

Statistical analysis was carried out on a microcomputer using the statistical software package, Systat (Systat Evanston, U.S.A.). The slope of the plant height and stem diameter growth curves were calculated over the period from June to August 1988 and May to August 1989. These slopes were analyzed as a randomized complete block design. The shoot and root weights and areas were analyzed as randomized complete block designs.

RESULTS AND DISCUSSION

The pattern of height increase over the two growing seasons is given in Figure 2 and the pattern of stem diameter increase is given in Figure 3. There were no significant differences in the slopes of the growth curves for either year. The seedlings grew very little in 1988 but showed strong growth in 1989.

Leaf number, leaf area, root area and shoot and root weight, leaf number are shown in Figures 4 to 7. There were no significant differences in any of these parameters. The pH 4.3 treated plants tended to show poorer growth than the other treatments. The pH 3.2 treated seedlings tended to have the highest shoot and root weights.

The seedlings treated with pH 3.2 SAR tended to grow better than the seedlings sprayed with the other two treatments. This may be due to fertilization by the additional nitrogen and sulphur inputs in this acidic treatment. Schulze (1989) has pointed out that the nitrogen inputs in acidic rain may initially have a stimulatory effect, but with time the acidification of the soil and loss of nutrient cations may lead ultimately a growth reduction. The soil pH was not measurably altered in the two years over which the experiment was conducted.

CONCLUSION

The establishment and growth of sugar maple seedlings on mineral soil was not adversely affected by highly acidic simulated acid rain applied for two growing seasons.

REFERENCES

Kuja, A., R. Jones, A. Enyedi. 1986. A mobile rain exclusion canopy system to determine dose-response relationships for crops and forest species. Water, Air and Soil Pollut. **31:** 307-315.

Kuja, A., Dixon, M. 1991. Effects of simulated acid rain on the growth, nutrition and physiology of sugar maple seedlings grown within a mobile rain exclusion canopy system. MOE/ARB report manuscript to be published.

Schulze, E.D. 1989. Air pollution and forest decline in a spruce (Picea abies) forest. Science **244:** 776-783.

Table 1. Physical and Chemical Characteristics of Soil Utilized.

Parameter	Plastic Lake Site (5-30 cm)
pH (water extr.)	5.1
pH ($CaCl_2$) extr.)	4.5
Percent Sand	37
Percent Silt	58
Percent Clay	5
Percent Organic Carbon	2.3
Ca[*] (me/100g)	0.66
Mg[*] (me/100g)	0.02
K[*] (me/100g)	0.02
Al[*]	0.54
Ca (mg/g)	2.2
Mg (mg/g)	1.3
K (mg/g)	0.43
Al (mg/g)	14.3
N (mg/g)	0.93
P (mg/g)	0.46
Fe (mg/g)	20.0
S (mg/g)	0.15
Cu (ug/g)	9
Ni (ug/g)	7
Pb (ug/g)	5
Cd (ug/g)	0.2
Zn (ug/g)	39

[*] NaCl extracted concentration

Table 2. Chemical composition of simulated acidic rain (SAR) solutions.

SAR[*] pH	H^+ ueq L^{-1}	Acid vol. ml L^{-1}	
		H_2SO_4	HNO_3
5.6	12	0	0
4.3	50	$1.001\ E^{-3}$	$8.849\ E^{-3}$
3.2	650	$1.301\ E^{-2}$	$1.151\ E^{-2}$

[*] All simulated rain solutions contained the following background ions (mg L^{-1}):

K - 0.063

Mg - 0.103

Na - 0.100

Cl - 0.023

Ca - 0.501

NH_4 - 0.744

NO_3 - 1.385

SO_4 - 2.771

S:N ratio = 2:1

Figure 1: Placement of Sugar Maples and Hybrid Poplars under Each Nozzle within the Rain Exclusion Canopy System

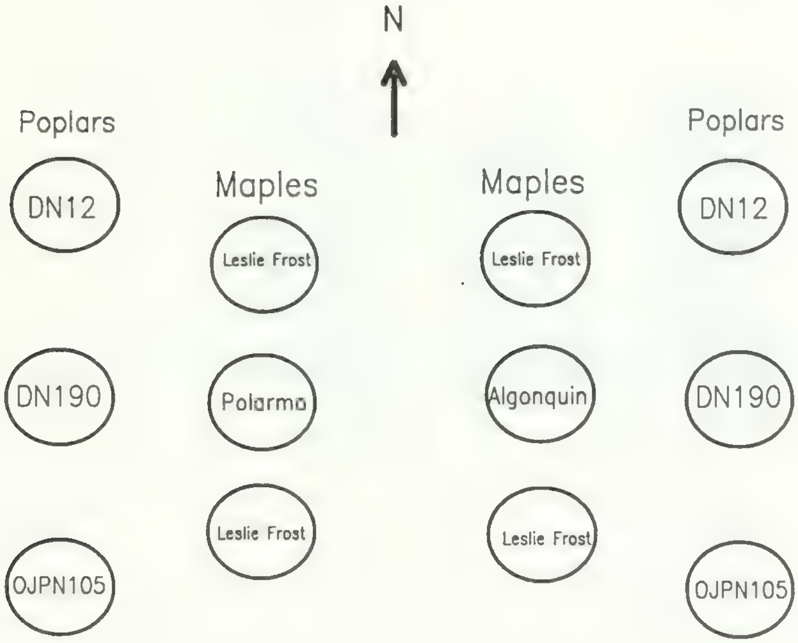


Figure 2: Height increase of Palermo seedlings exposed to simulated acid rain for two growing seasons

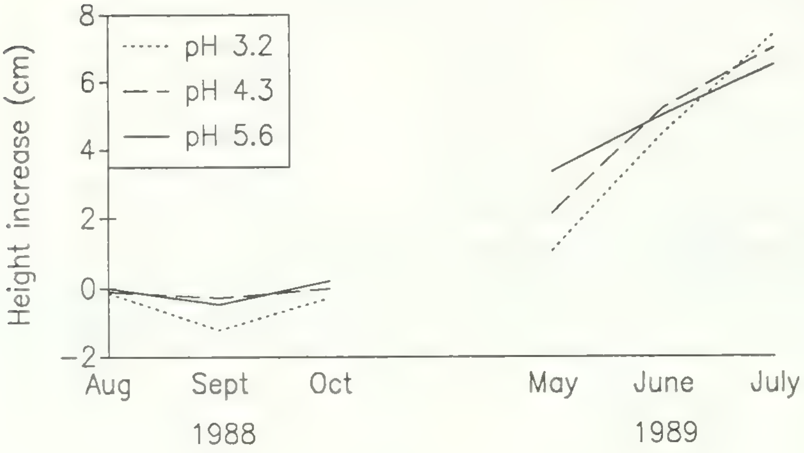


Figure 3: Stem diameter increase of Palermo seedlings exposed to simulated acid rain for two growing seasons

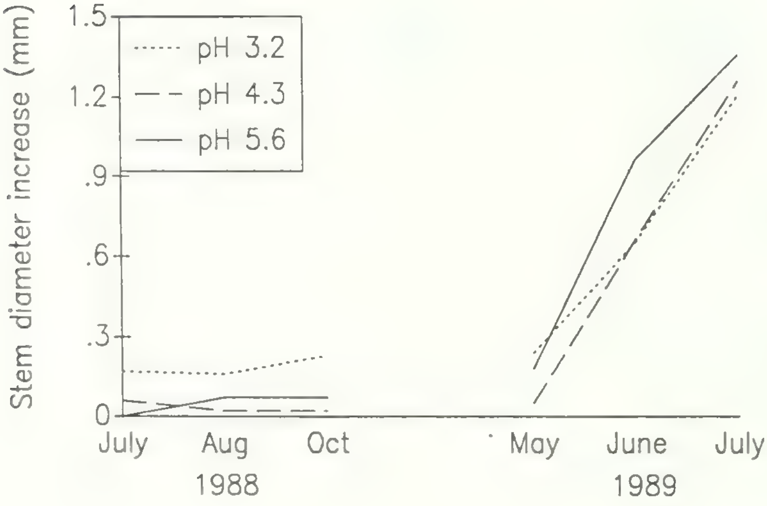


Figure 4: Average dry weight of roots and shoots of Palermo sugar maple seedlings exposed to simulated acid rain for 1.5 growing seasons

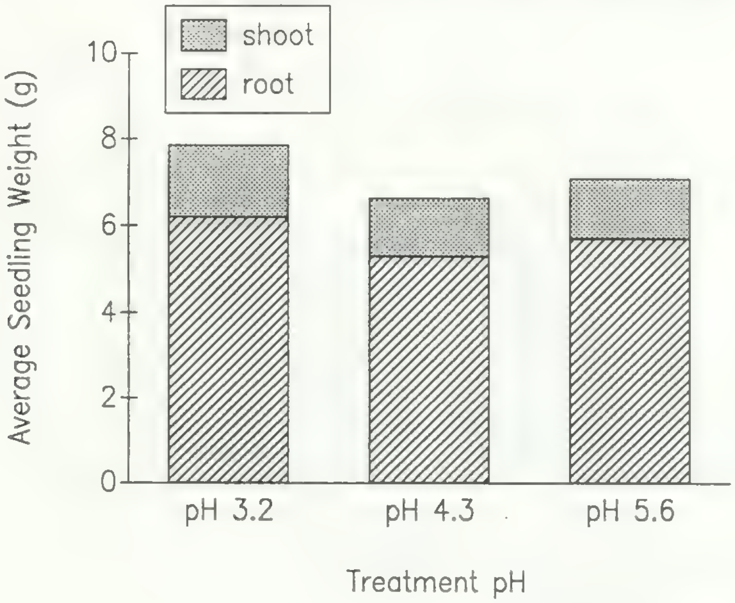


Figure 5: Average number of leaves per plant of Palermo sugar maple seedlings exposed to simulated acid rain for 1.5 growing seasons

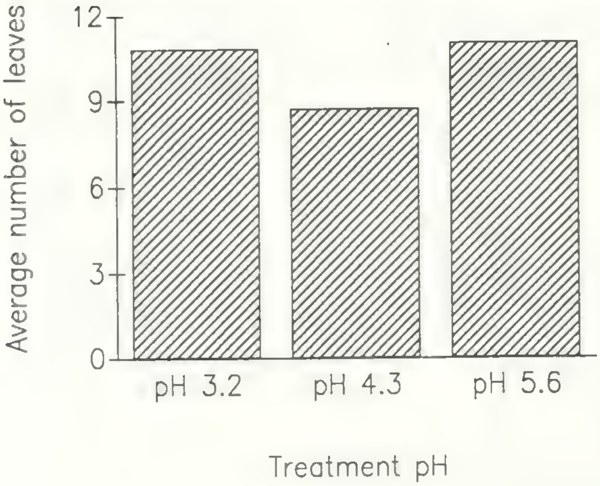


Figure 6: Average leaf area of Palermo sugar maple seedlings exposed to simulated acid rain for 1.5 growing seasons

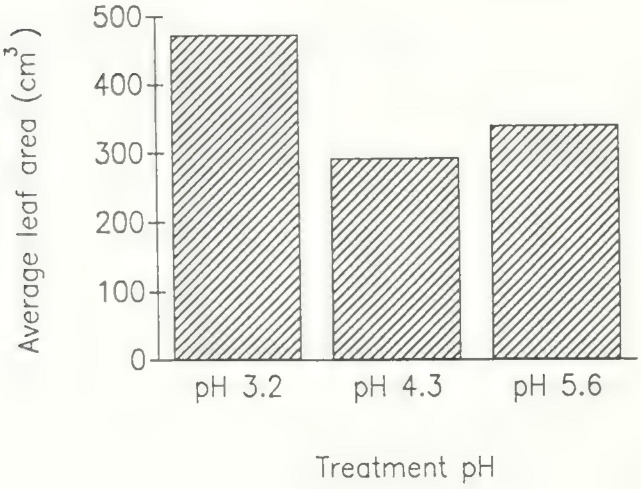
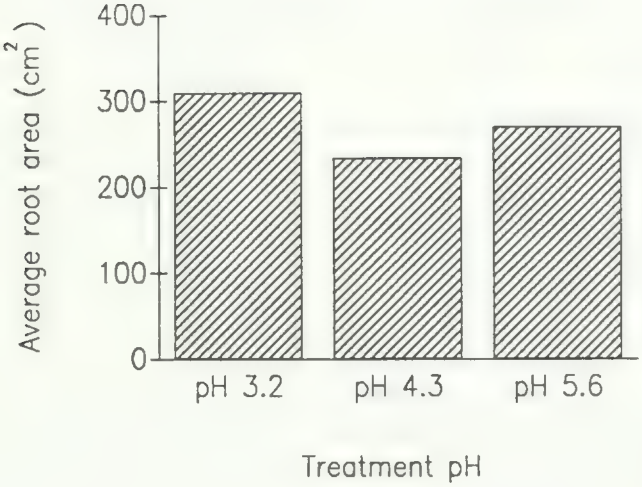


Figure 7: Average root area of Palermo sugar maple seedlings exposed to simulated acid rain for 1.5 growing seasons



THE EFFECT OF SIMULATED ACID RAIN ON ROOT GROWTH OF SUGAR MAPLE SEEDLINGS

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INTRODUCTION

Sugar maples (Acer saccharum Marsh.) have over the past 6 years been suffering a decline which is most evident in Quebec and the northeastern United States. The cause is as yet unknown, although insect defoliation, drought and air pollution have been implicated. All these factors could result in a significant affect on root growth. Defoliation not only reduces the quantity of photosynthates translocated to the roots but also draws on carbohydrate reserves during refoliation. Reduced root growth, particulary the fine roots, will decrease the soil volume accessible to the tree, which in times of drought may be critical to the tree's ability to maintain its water status.

The roots of sugar maples grow more slowly than other hardwood species. Webb (1977) found that sugar maple seedlings regenerated substantially fewer new roots than did silver maple or white ash. This may in part explain why transplanting of sugar maples is more difficult than for these species. Dumbroff and Webb (1978) note that the survival and growth of sugar maple seedlings planted on abandoned farmland in Eastern Canada has been unsatisfactory. They attribute this, in part, to insufficient root establishment before rapid shoot growth.

The slow growth and sensitivity of the root system of sugar maple may be of key importance to its present decline. Research in the Solling area of the Federal Republic of Germany has focused on root damage to European beech and Norway spruce (Matzner and Ulrich 1985, Matzner, Murach and Fortmann 1986). Matzner and Ulrich (1985) attribute drought and soil acidification, coupled with the mobilization of aluminum and death of fine roots, to the decline observed in this part of Germany. In North America, Thornton, Schaedle and Raynal (1987) investigated the effect of aluminum on the growth of sugar maple roots in solution culture. They found no significant difference ($p=0.05$) of mean root growth increment, after six weeks, between maple roots growing in 2000 uM Al at pH 4.0 and the control solution at pH 4.0. The roots at intermediate aluminum concentrations, 500 and 1000 uM Al, showed a significant stimulation of root growth.

Experiments on the effect of simulated acid rain on sugar maple root growth have shown adverse effects at pH 3.0 (Raynal et al., 1982; Dustin and Raynal, 1988). These were short term experiments, 1 to 2 months, designed to study the acute effects of acidity rather than more subtle long term effects caused by alterations in soil chemistry. They found no adverse effects above this pH. The effects noted by Dustin and Raynal (1988) were not evident after an initial 10 day period of seedling growth. Although there are examples of researchers specifically investigating root growth in relation to atmospheric pollution, most research has focused on effects on shoots, due to the inherent difficulties in measuring roots.

This experiment was designed as a pilot experiment to assess the suitability of root window chambers in the study of sugar maple seedling roots and to study the effect of soil acidification, through the application of simulated acid rain, on spring root flush.

MATERIALS AND METHODS

Sugar maple seedlings and soil from the A and B horizons of a humo-ferric podzol were collected on November 12, 1987 from the Swan Lake research centre in Algonquin Provincial Park, Ontario. Table 2 gives the soil chemistry. The seedlings were 20 - 25 cm high and removed from the native site in 30 cm diameter soil cores to minimize root disturbance. Thirty root chambers were constructed by 25 November, 1987 and filled with soil to represent a reconstructed soil profile (Figure 1). One of the Plexiglass plates was carefully removed to expose the soil profile. The seedling roots were carefully washed from the soil cores and spread onto the soil profile. The plexiglass plate was replaced and the chambers were put in a specially constructed containment box in the refrigerator at 4 °C. Seedling chambers were randomized among 3 simulated acid rain treatments of pH 5.6, 4.3 or 3.2, the compositions of which are given in Table 1. Each treatment had 10 replicate chambers. Rain solutions were poured onto the central area of the chambers using a graduated cylinder approximately once a month when the seedlings were dormant, and more often when they had broken dormancy and required more water (Table 3).

The maples showed signs of bud break by the 16 March, 1988 and had started to leaf out by the 20 April, 1988 at which time they were transferred to the greenhouse. The windows of the chambers were not covered while in the refrigerator since they were continuously in the dark. However, in the greenhouse, they were covered with a black cloth and kept in a painted containment box.

The seedlings were harvested on 16 May, 1988. Shoots were removed and the root chambers opened. Soil was then carefully washed from the seedling roots and the roots were separated into white root and brown sections. All plant parts were dried at 60 °C for 48 hours and then weighed. Statistical analyses were carried out on a microcomputer using the statistics package Statgraphics 2.1 (STSC Plus*Ware, Maryland, U.S.A.).

RESULTS

There were no significant differences in shoot height, shoot weight, number of leaves, or weight of white or brown roots among treatments. The seedlings tended to be tallest in the pH 5.6 treatment and shortest in the pH 3.2 treatment (Figure 2). Similarly, there were more leaves in the pH 5.6 treatment than in the pH 3.2 (Figure 3). However, the shoots of the pH 5.6 treated maples weighed less than the pH 3.2 (Figure 4). Figure 5 shows both white and brown root weight. These were both lowest in the pH 5.6 treatment and highest in the pH 3.2. Root to shoot ratios were close to 1:1 in all treatments (Figure 6). Comparison of white root weight with brown root weight shows a very poor correlation (Figure 7), similarly there was a poor correlation between shoot weight and white root weight (Figure 8). Chemical analysis of the soil leachates taken at three times during the experiment showed elevated calcium and magnesium concentrations in the most acidic, pH 3.2 solution, leachate (Figure 10 and 11). Potassium concentrations were similar among treatments (Figure 9). Sulphate concentration was much higher in the pH 3.2 leachate compared with the other treatments (Figure 12). Nitrate values were similar among treatments but highest in the pH 3.2 treatment (Figure 13). In all treatments, there was an increase in elemental concentration in the leachate as the experiment progressed.

DISCUSSION

Root growth can be investigated by several different methods which are explained in detail by Bohm (1979). The root chamber method was chosen because it allows observation of root growth during the experiment, as well as harvest of the total root system at the end of the experiment. Unfortunately, very few roots were visible through the plexiglass panes and, therefore, no measurement of growth by this method was attempted.

Root growth in maples usually displays a periodicity. Morrow (1950) reports that up to 80% of the total years root growth in sugar maple occurs from mid March to late June. It is unclear whether this is under endogenous control or whether it is a response to environmental conditions. Morrow (1950) suggests that probably both are involved. Roots will grow when conditions are suitable, yet the main flush occurs in the spring and not equally in the autumn even when soil moisture and temperature conditions are comparable. The winter months are a period of dormancy with little or no growth occurring (Lyr and Hoffmann 1967). Richardson (1958) found in silver maple (Acer saccharinum) that new root growth resumed when the buds had broken their physiological dormancy and the temperature had increased above 5 °C. Webb (1977), in a greenhouse experiment, measured root growth in silver maple and sugar maple in December before the buds had lost their physiological dormancy. However, the main period of root growth was from March to May.

Dumbroff and Webb (1978) maintain that the roots of sugar maple will continue to grow except when the soil is frozen. Sugar maple seedlings, in this experiment, were kept at 4.5 °C for approximately 5 months to simulate winter conditions. During this period no root growth was detected through the plexiglass root windows in any root chamber. White root growth was visible by late April when the chambers had been moved to the greenhouse.

The white root growth measured was probably produced within a month prior to harvest for two reasons: 1) root growth is very low below 10 °C and reaches a maximum usually between 20 and 30 °C (Ladefoged 1939), and 2) Engler (1903) found that the root tips of hardwoods turn brown in 3 to 6 weeks (Morrow 1950). Since the size and shape of roots appeared very similar among the chambers, root weight would probably give a similar picture to root length or surface area. The white, new root weight (Figure 5) shows a trend toward increased root growth in the pH 4.3 and 3.2 treatments compared with to the pH 5.6 treatment.

A regression was performed comparing shoot weight with white root weight (Figure 7), since the pH 5.6 treated plants were, by chance, slightly smaller than the other treatments (Figure 4), which might have affected root growth. The poor correlation suggests that for this experiment, initial size of seedlings was not an important factor influencing new root growth. Brown root weight was also compared to new white root growth. Intuitively, one might expect a good correlation between the amount of old roots and production of new roots. Figure 8 illustrates that this is not the case and that, as with shoot weight, the size of the plant is not an important factor influencing root growth. The mean white root weight of the seedlings grown in the pH 3.2 treatment was considerably greater (1.91 times) than that in the pH 5.6 treatment. Although not statistically significant, this does suggest a shift in carbon allocation toward the roots in the pH 3.2 treatment.

Alterations in carbon allocation are often used as indicators of stress. Waring and Schlesinger (1985) note that nutritionally-stressed Scots pines allocate more than 60% of their photosynthate to their roots whereas non-stressed trees allocate only 40 %. The greater height and number of leaves in the pH 5.6 treatment compared to the greater root growth in the pH 3.2 treatment may be an indicator of acid stress. A shift in allocation can be reflected in changes in the root to shoot ratio. Stresses such as soil dryness, nutrient stresses or high temperatures can increase the root to shoot ratio whereas shading, nitrogen fertilization, higher air temperatures and sufficient soil moisture can induce a decrease in the root to shoot ratio (Lyr and Hoffmann 1967). Figure 6 shows no significant alteration in the root to shoot ratio due to acid rain treatment. Since the new white root is only a small part of the total root mass, it will have little effect on the ratio and therefore, for short term experiments, it is an insensitive indicator of stress. Root growth is almost entirely from carbohydrate reserves, since it occurs before the emergence of the leaves. Therefore, the indication of increased new root growth in the pH 3.2 treatment is probably due to direct stimulation of root formation by the acidity.

Acidic deposition may affect many components of a forest ecosystem. One area of particular interest is the alteration in soil chemistry. Tamm and Hallbacke (1986) have found that the soil pH in southern Sweden has, over the past 50 years, decreased by up to 0.9 of a pH unit and that the pH of the C horizon has decreased often to within the aluminum buffering range (pH 4.5). They attribute the acidification of the C horizon to acid deposition. The A horizon of the Algonquin soil is already within this range and a 0.4 decrease in the pH of the B horizon would also put it within this range (Table 2). Aluminum mobilization and the resultant death of fine roots has been implicated in the decline of European beech in the Solling area of west Germany (Matzner and Ulrich 1985). Aluminum was not analyzed in the leachate samples because of the instability of aluminum complexes and the delay in time between collection and analysis. Thornton et al. (1986) have shown that aluminum solution concentrations below 2000 uM may not significantly decrease root growth in sugar maple seedlings. He found a stimulation in root growth at lower aluminum concentrations. A more important aspect may be the loss of nutrients such as calcium, magnesium and potassium due to increased soil leaching.

Roberts et al. (1988) state that poor root growth in declining Norway spruce and Silver fir trees appears to result from reduced carbohydrate translocation from magnesium deficient needles rather than aluminum toxicity resulting from soil acidification. Foliar deficiencies reflect the soil nutrient status which may be upset through leaching of nutrient cations. Figures 10 and 11 suggest that more calcium and magnesium are being leached from the soil by the most acidic treatments compared with the control (pH 5.6). The greatest leaching of potassium (Figure 9) is also in the pH 3.2 treatment but the pattern is less clear. This may be because potassium is highly mobile and readily leached over a wide range of acidities. This leaching of nutrient cations could result in nutrient deficiencies if the plants are at sub-optimal nutrient levels. The maple foliage was not analyzed so this is not known. However, the Algonquin soil is a nutrient poor soil as reflected in the root to shoot ratio of 1:1 (Figure 6) as compared with a ratio of 1:2 on better soils (Lyr and Hoffmann 1967).

Higher nitrogen and sulphur inputs in the more acidic treatments could have a fertilizer effect on the seedlings. Enyedi and Kuja (1986) found a stimulation in the growth of corn with increasingly acidic rainfall. Figure 12 reflects the sulphate concentration of the acid rain solutions applied (Table 1). Figure 13 does not reflect the nitrate input, thus suggesting that the nitrate is being largely retained in the soil. This pattern was found also by Stuanes (1983), investigating the effect of acid rain on forest growth in Europe. He found only 30% of the nitrogen input from precipitation was collected in ground water run-off compared with about 100% of the sulphate. The nitrogen fertilization may increase shoot growth in time and lead to an alteration in the root to shoot ratio. It may also exacerbate other deficiencies such as magnesium by simulating growth and demand for these nutrients. A lower root to shoot ratio can also be detrimental in periods of drought stress or if the roots are damaged by mobilized aluminum. This experiment was too short in duration to be able to determine the implications of these shifts in soil chemistry. It would be necessary to conduct the experiment for at least one year in order that growth changes reflect changes in photosynthesis rather than simply allocation of stored carbohydrates. Future experiments should be conducted with sufficient replicates that a few pots could be harvested once a month. This method could provide a clear picture of root growth without the problems of soil alteration and disturbance inherent in other methods of root measurement.

CONCLUSIONS

The results of this pilot experiment show that root window chambers were not an asset in measuring sugar maple root growth since few roots grew along the plexiglass. New root growth tended to be greater in the pH 3.2 treatment compared to the other two treatments. This may not be an indicator of stress since spring root and shoot growth is primarily, if not entirely, from stored carbohydrates rather than current photosynthate. Acidification of the soil, loss of the calcium and magnesium, and the accumulation of nitrate may, in time, result in damage to the root system and could be a factor in the decline of sugar maples.

REFERENCES

Bohm, W. 1979. Methods of studying roots. Springer-Verlag, Berlin.

Dustin, C.D. and Raynal, D.J. 1988. Effects of simulated acid rain on sugar maple seedling root growth. Envir. and Exp. Bot. 28(3): 207 - 213.

Dumbroff,E.B. and Webb, D.P. 1978. Physiological characteristics of sugar maple and implications for successful planting. For. Chronicle 54(2): 92 -95.

Enyedi, A.J. and Kuja, A.L. 1986. Assessment of relative sensitivities during early growth stages of selected crop species subjected to simulated acid rain. Water, Air and Soil Pollut. 31: 325-335.

Ladefoged, K. 1939. Investigations about the periodicity in eruption and length growth of roots of some of our commonest tree species. Translated by D.C.F. Fayle. Andr. Fred Host and Son, Kopenhagen.

Lyr, H, and Hoffmann, G. 1967. Growth rates and growth periodicity of tree roots. IN: Romberger, J.A. and Mikola, P. Internat. Review For. Res. Vol.2, Academic Press, New York.

Matzner, E. and Ulrich, B. 1985. "Waldsterben": Our dying forests. Experientia 41(3): 578 - 584.

Matzner, E., Murach, D. and Fortmann, H. 1986. Soil acidity and its relationship to root growth in declining forest stands in Germany. Water, Air and Soil Pollut. 31: 273 - 282.

Morrow, R.R. 1950. Periodicity and growth of sugar maple surface layer roots. J. For. 48: 875 - 881.

Raynal, D.J., Roman, J.R., and Eichenlaub, W.M. 1982. Response of tree seedlings to acid precipitation . II. Effect of simulated acidic canopy throughfall on sugar maple seedling growth. Envir. and Exp. Bot. 22(3): 385 - 392.

Richardson, S.D. 1958. Bud dormancy and root development in Acer saccharinum. In: The Physiology of Forest Trees. K.V. Thimann (ed.), p. 409 - 425. Ronald Press, New York.

Roberts, T.M., Skeffington, R.A. and Blank, L.W. 1988. Causes of Type 1 spruce decline in Europe. in press: Central Electricity Research Laboratories, Surrey.

Stuanes, A.O. 1983. Possible indirect long-term effects of acid precipitation on forest growth. Aquilo Serie Botanica 19: 50 - 63.

Tamm, C.O. and Hallbacke, L. 1986. Changes in soil pH over a 50 year period under different forest canopies in SW Sweden. Water, Air and Soil Pollut. 31: 337 - 341.

Thornton, F.C., Schaedle, M. and Raynal, D.J. 1986. Effects of aluminum on the growth of sugar maple in solution culture. Can. J. For. Res. 16: 892-896.

Waring, R.H. and Schlesinger, W.H. 1985. Forest Ecosystems: concepts and management. Academic Press Inc. Orlando. 340 pp.

Webb, D.P. 1977. Root regeneration and bud dormancy of sugar maple, silver maple, and white ash seedlings: effects of chilling. For. Sci. 23(4): 174 - 183.

Table 1: Chemical composition of simulated acid rain solutions applied to sugar maple seedlings

pH	3.2	4.3	5.6
Ca (mg/L)	.40	.40	.41
Mg (mg/L)	0.15	.15	0.15
K (mg/L)	0.115	.10	0.11
NO_3 (mg/L)	3.18	.54	0.23
SO_4 (mg/L)	28	4.90	2.35

Table 2: Chemical composition of the Algonquin soil

	A horizon	B horizon
pH (water)	4.1	4.9
pH ($CaCl_2$)	3.4	4.2
Ca (ug/g)	1.3	0.4
Mg (ug/g)	0.18	0.05
K (ug/g)	0.11	0.06
CEC	6.5	3.8
Organic C (%)	6.3	5.4
S (%)	0.034	0.27
N (mg/g)	3.5	2.1

Table 3: Date and quantity of simulated acid rain application

Date	Quantity (ml)
17 Dec. 87	50
22 Dec. 87	50
31 Dec. 87	50
11 Jan. 88	25
4 Feb. 88	25
4 Mar. 88	25
18 Mar. 88	50
5 Apr. 88	50
20 Apr. 88	50
26 Apr. 88	50
10 May 88	75
Total	500

Figure 1:

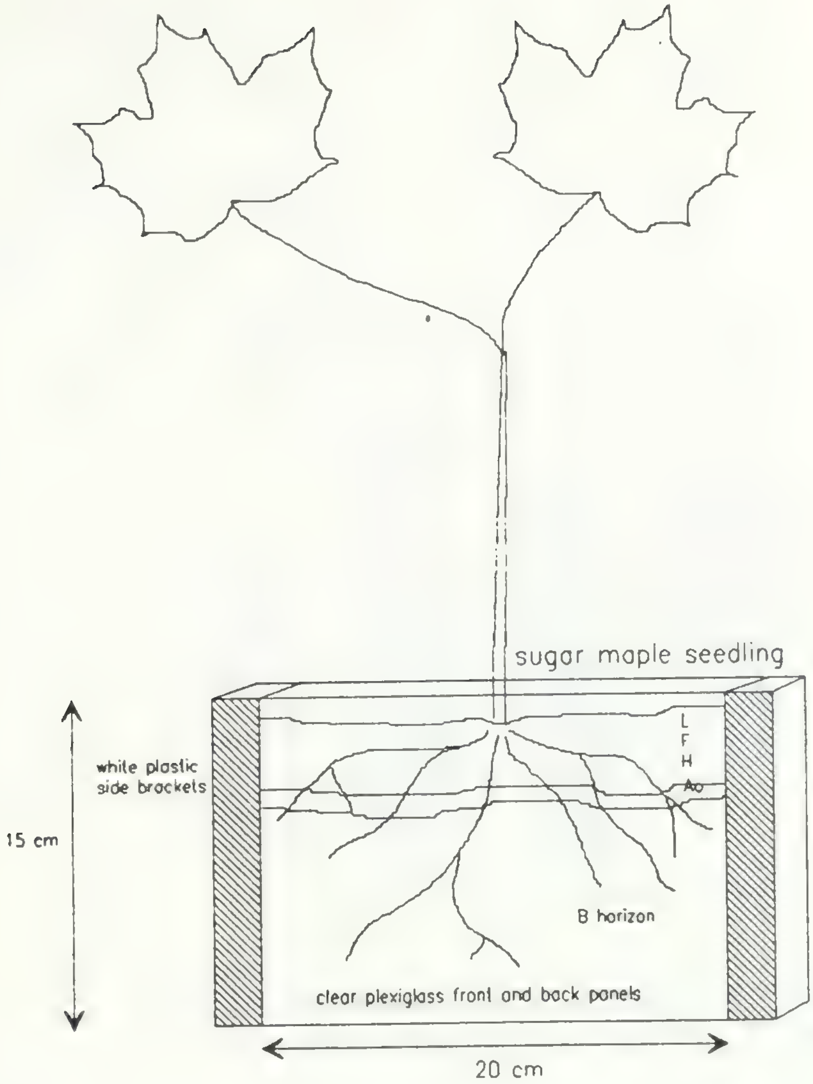
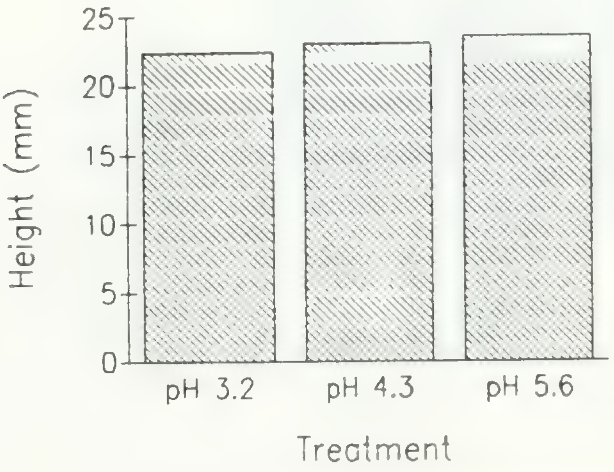

sugar maple seedling
L
F
H
Ao
white plastic side brackets
15 cm
B horizon
clear plexiglass front and back panels
20 cm

Figure 2: Height of Sugar Maple Seedlings after Exposure to Simulated Acid Rain for 5 months



columns are not significantly different ($p>0.05$)

Figure 3: Number of Leaves on Sugar Maple Seedlings Exposed for 5 months to Simulated Acid Rain

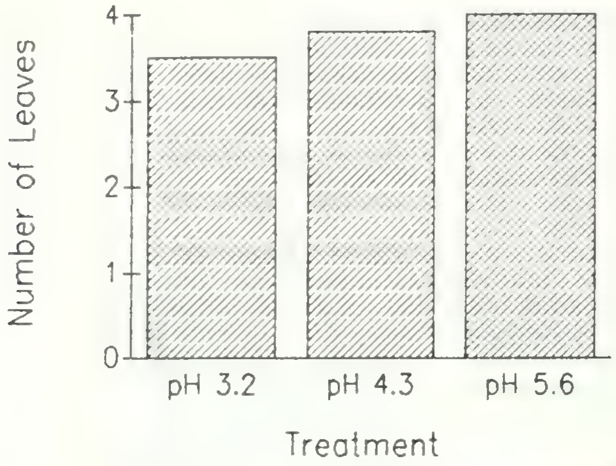


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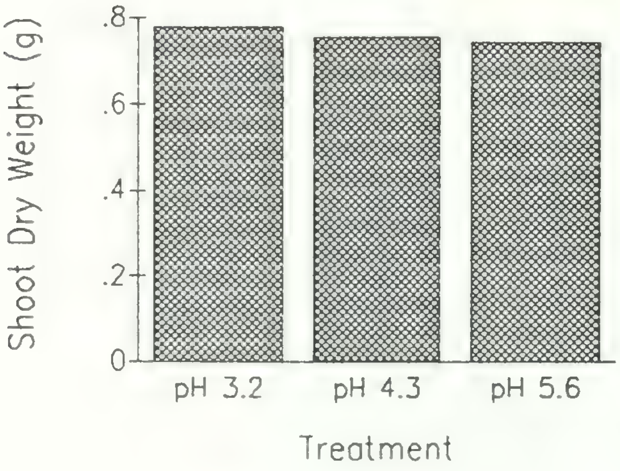
Figure 4: Shoot Weight of Sugar Maple Seedlings Exposed for 5 months to Simulated Acid Rain



columns are not significantly different (p>0.05)

Figure 5: Root growth of sugar maple seedlings exposed to simulated acid rain for five months

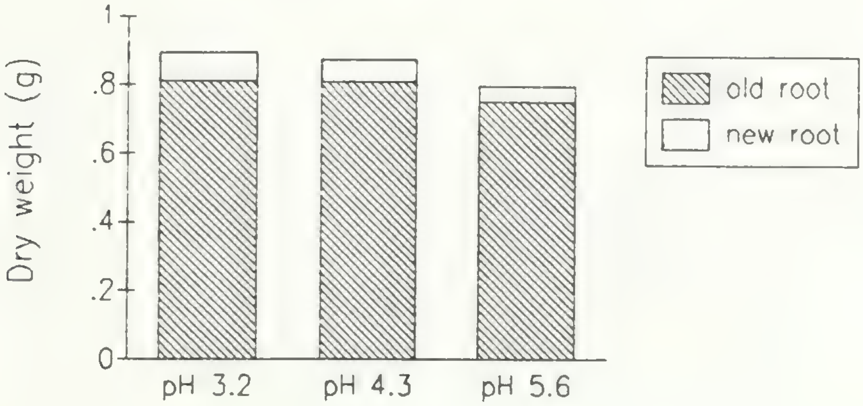
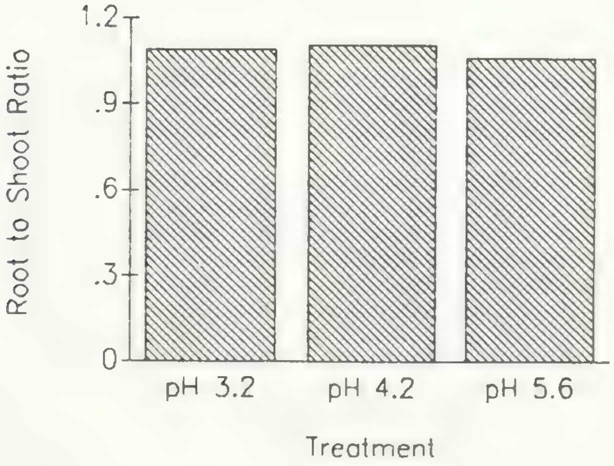


Figure 6: Root to Shoot Ratio of Sugar Maple Seedlings Treated for 5 Months with Simulated Acid Rain



columns are not significantly different ($p > 0.05$)

Figure 7: White root weight of sugar maple seedlings exposed to SAR solutions for 5 months compared to brown root weight

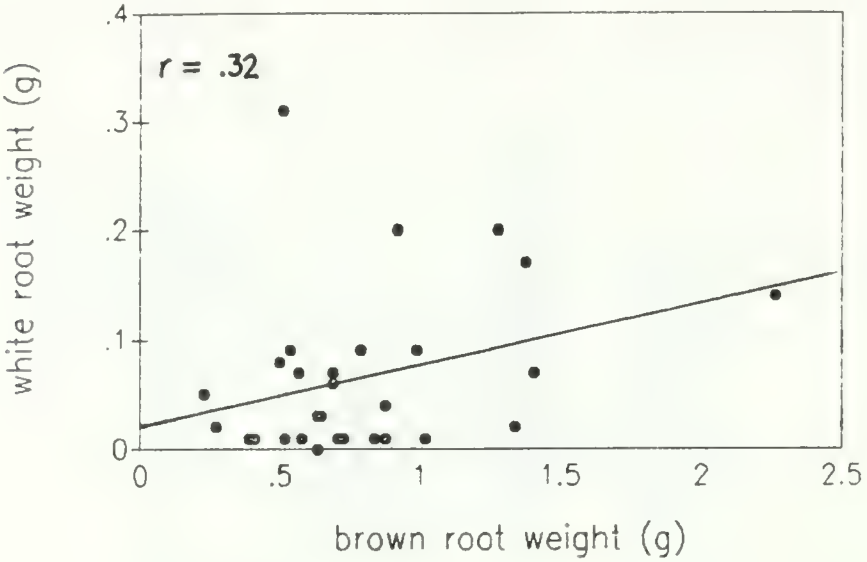


Figure 8: White root weight of sugar maple seedlings exposed to SAR solutions for 5 months compared to shoot weight

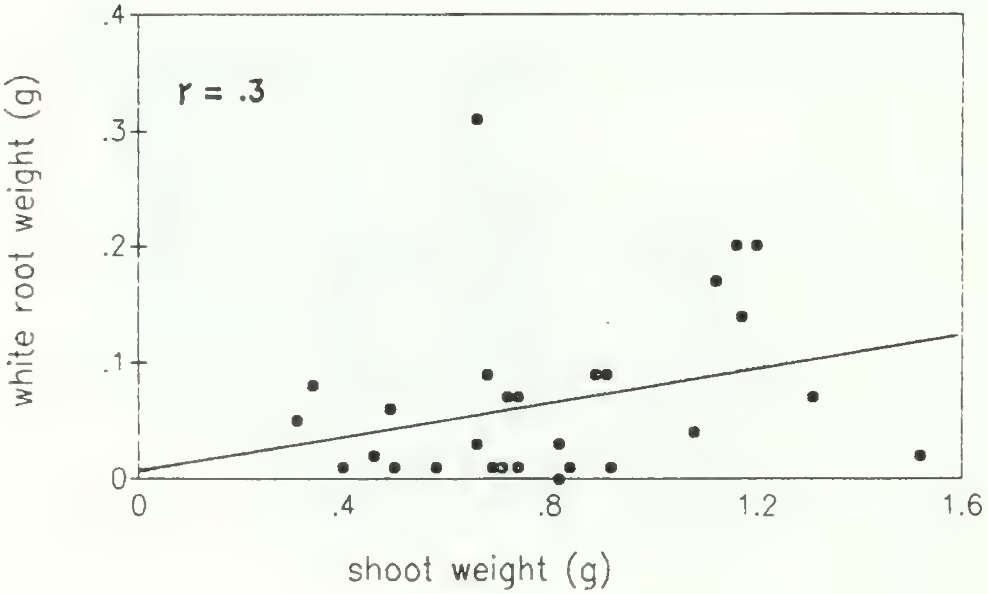


Figure 9: Potassium concentration of soil solutions leached from root chambers containing sugar maple seedlings

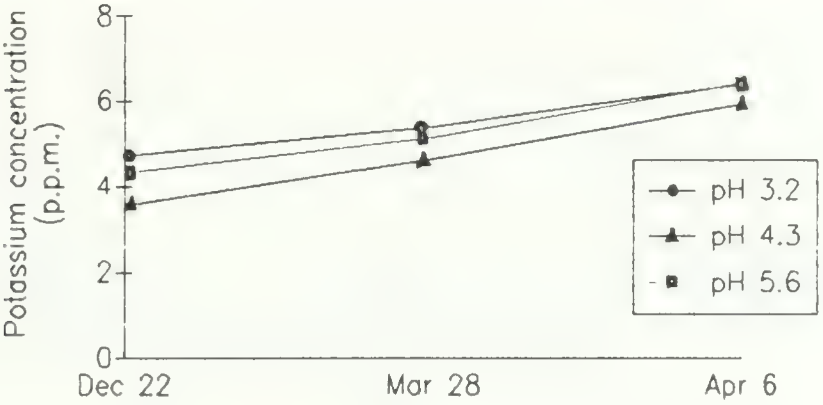


Figure 10: Calcium concentration of soil solutions leached from root chambers containing sugar maple seedlings

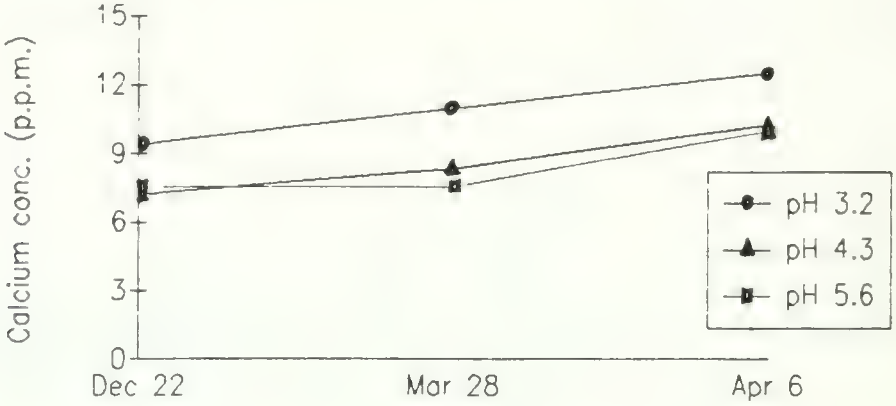


Figure 11: Magnesium concentration of soil solutions leached from root chambers containing sugar maple seedlings

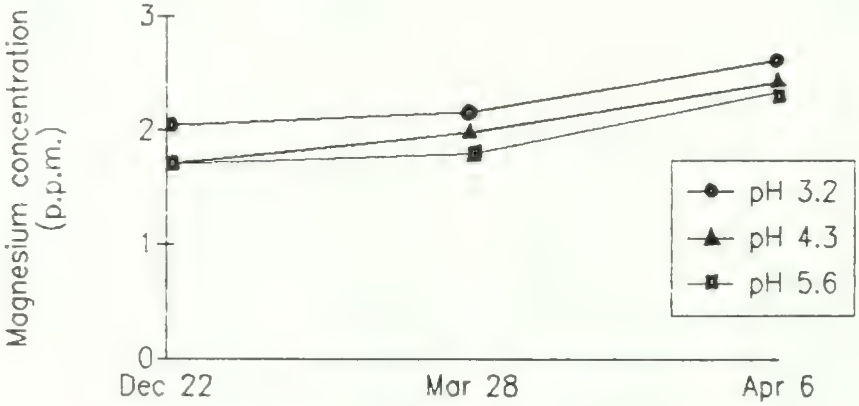


Figure 12: Sulphate concentration of soil solutions leached from root chambers containing sugar maple seedlings

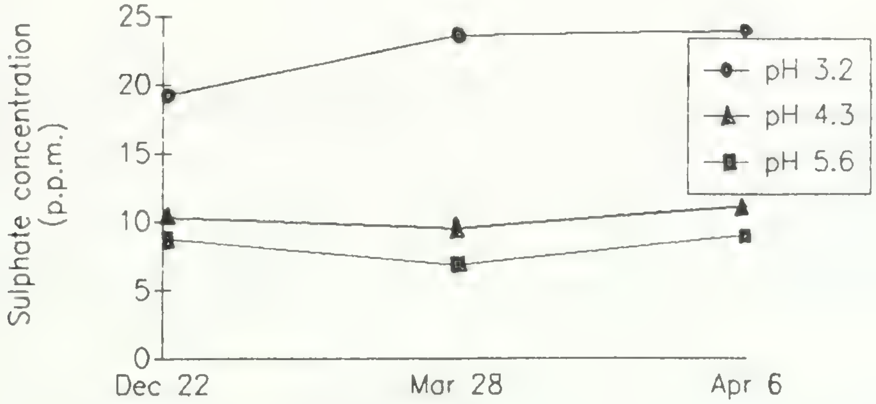
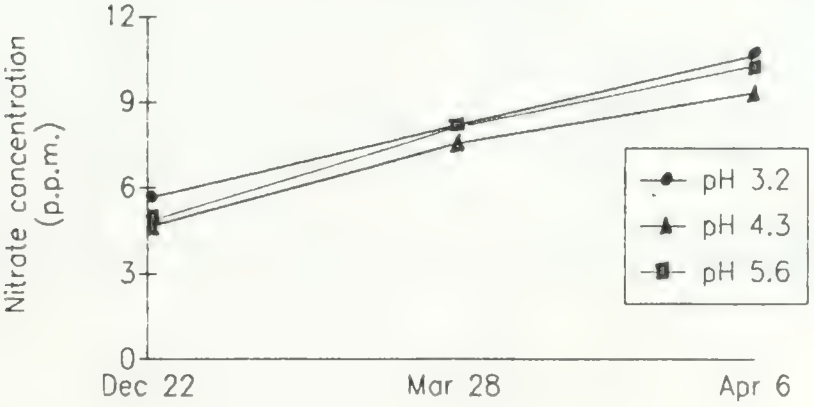


Figure 13: Nitrate concentrations of soil solutions leached from root chambers containing sugar maple seedlings



EFFECTS OF SIMULATED ACIDIC RAIN ALONE AND IN COMBINATION WITH OZONE ON THE GROWTH AND NUTRIENT STATUS OF WHITE SPRUCE (Picea glauca (Moench) Voss.) SEEDLINGS

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INTRODUCTION:

In recent years, ozone and acidic precipitation have both been implicated as important contributors to the observed decline of coniferous forests in Europe and North America (Ulrich et. al. 1980; Ulrich, 1983; Johnson, 1983; Hornbeck and Smith, 1985; Linzon, 1985; Van Breemen, 1985). For example, researchers have postulated that the decline of Norway spruce (Picea abies L.) in southwest Germany is due to ozone and acid rain. Ozone causes physiological and anatomical damage to spruce foliage which is then susceptable to foliar leaching of easily-mobilized nutrients, such as Mg, by acid rain or fog (Zottl et. al. 1986; Krause, 1988).

In North America, a decline of red spruce (Picea rubens Sarg.) has been observed for the past 25 years in high elevation forests of New York, Vermont, and New Hampshire under circumstances indicative of stress-related disease (Siccama et. al. 1982; Johnson, 1983; Vogelmann and Rock, 1988). Jagels (1986) described red spruce decline in Maine and showed that decline symptoms occurred most frequently on trees growing on thin mountain soils on granite bedrock where ozone levels are elevated (e.g. up to 0.14 ppm) and fog pH values are low (e.g. pH 2.9). Decline symptomology consisted of chlorosis of adaxial needle surfaces, needle loss (oldest to youngest) and development of latent vegetative shoots of dorsal surfaces of declining branches (Jagels, 1986). Weinstein et. al. (1987) also suggested that red spruce decline in the northeast U.S.A. may be linked to the widespread occurrence of ozone and acidic rain or fog.

White spruce (Picea glauca (Moench) Voss), is a wide-ranging tree species in the north temperate and boreal forests of North America. It is common throughout southern Ontario, which is currently subjected to both ozone and acidic precipitation. Ulrich (1981) proposed that soil acidification from acidic precipitation causes a loss of essential plant nutrients from soils, as well as mobilization of toxic ions like aluminum. Studies suggest that white spruce seedlings are sensitive to aluminum (Nosko et. al. 1988; Hutchinson et. al. 1986). Also, Percy (1986) demonstrated that juvenile white spruce seedlings are sensitive to increased rain acidity (pH<4.6). Therefore, increased soil acidification from acidic precipitation, in combination with ambient ozone, may inhibit the growth and establishment of white spruce in Ontario, especially on the thin mineral soils of the Canadian Shield.

An indoor study was carried out at the MOE Phytotoxicology controlled environment laboratory in Brampton, Ontario to investigate the potential interactive effects of simulated acidic rain (SAR) and controlled ozone fumigations on growth and nutrient status of greenhouse-grown, white spruce seedlings. This study was conducted in order to provide preliminary results for an ongoing, multi-year, field study. At present, a mobile rain exclusion canopy (REC) system is being used to determine the impact of controlled applications of SAR, alone and in combination with ozone, on the growth and physiology of potted white spruce seedlings established in field plots.

MATERIALS AND METHODS:

1. Plant Culture

Two-year-old white spruce seedlings were obtained from the Ministry of Natural Resources nursery at Midhurst, Ontario. The seedlings were bare-root transplanted into six-litre, plastic pots containing 2mm-screened brunisol soil on October 14, 1987. This mineral soil was obtained from the B horizon of a soil site at Plastic Lake located near the town of Dorset in the Muskoka district of Ontario. The chemical and physical composition of this soil are shown in Table 1.

Potted seedlings were maintained in a garden plot (buried to within 2 cm of pot rims) until January 18, 1988. At this time, the potted seedlings were transferred to the greenhouse. The greenhouse was equipped with automatic temperature and humidity controls and was supplied with charcoal-filtered air to reduce ambient gaseous pollutant levels. All seedlings were placed on elevated benches and pot positions were randomized after each treatment application. Day/night temperature was maintained at 22/18°C and humidity was set at 60% ± 10%. Fluorescent and incandescent lamps were used to extend the photo period to 14 hours and produced a photon flux density of 350 umol $m^{-2}s^{-1}$. All pots were irrigated with deionized water as required.

2. Treatment Chemistry and Application

Ten replicate test plants were subjected to each of six treatments. These were:

a) pH 5.6 (12 ueq H^+L^{-1}) SAR
b) pH 4.3 (50 ueq H^+L^{-1}) SAR
c) pH 3.2 (650 ueq H^+L^{-1}) SAR
d) pH 5.6 SAR and Ozone
e) pH 4.3 SAR and Ozone
f) pH 3.2 SAR and Ozone

SAR solutions contained mineral salts and acids (H_2SO_4 and HNO_3) based on ambient rain chemistry in southern Ontario (Chan et. al. 1983) and had a final S:N mass ratio of 2:1 (Table 2). Control plants were irrigated with deionized water only.

SAR was applied to test plants in four indoor rain chambers. The four chambers had the following dimensions; 7.3 m height, 1.2 m width and 1.2 m depth. To ensure uniform SAR distribution, all plants were placed on a turntable (1.1 m diam.) located at the base of each chamber. Each chamber compartment was illuminated by a 1000 watt multi-vapour lamp mounted at the top of each chamber. Photon flux density at table level was 50 +/- 5 umol $m^{-2}s^{-1}$ during each SAR application.

SAR was introduced into each chamber from a single nozzle (Bete Fog Nozzles Inc., Model SRN303) centred 5.3 m above each turntable. Each nozzle produced water droplets (1mm median diam.) at a maximum application rate of 1.2 cm hr^{1}. Events of lower intensity were achieved by reducing rotometer flow and by activating on/off timers to produce intermittent treatment application for a designated period.

All potted white spruce seedlings were maintained on greenhouse benches until buds began to break dormancy. Needle flush commenced approximately three weeks after the plants were brought indoors. A schedule of treatment applications and measurements is shown in Table 3. SAR treatment applications began on February 10, 1988 and continued until April 18, 1988. During this period, SAR was applied to the test plants three times per week (Mon, Wed, Fri). Treatments were not applied during the week of April 7 to 14. For each SAR event, a total of 8mm of SAR was applied intermittently, in a 3 hour period, by utilizing timers set on a 6 min on/off cycle. The total SAR applied over this study period was 210 mm. Treatments were rotated amongst the four chambers each day and chamber temperatures and humidities were recorded before and after each event.

Beginning on February 11, 1988, seedlings designated for exposure to ozone were fumigated on Tuesday of each week in one of two fumigation chambers. Ozone fumigations were conducted until April 19 (plants were not fumigated during the week of April 7 to 14). The duration of each ozone event was 7 hours with a mean concentrations of 0.062 ppm ozone. Each fumigation began with an initial ozone concentration of 0.02 ppm and peaked at 0.11 ppm after 5 hours. The ozone concentration was reduced gradually to zero at the end of 7 hours.

Ozone fumigations were controlled automatically by a microcomputer linked directly to a mass flow controller (Matheson Model 8259 multiple dyna blender) and a U.V. photometric ozone analyzer (Thermoelectron Model 49, Hopkinton, Mass.). Ozone was generated using a generator (Griffin Techniques Corp. Model GTC.25 Lodi, N.J.). Teflon tubing (0.25 in. O.D.) was used between all components in the chamber system. Chamber conditions were kept at 20 to 22°C and humidity was maintained at 75% R.H. during fumigations. Plants which were not designated for exposure to ozone were placed in a second chamber at identical temperature and humidity for control purposes. Light levels in both chambers were maintained at 420 $umolm^{-2}s^{-1}$.

3. Measurements and Observations

Plant height (cm), basal stem diameter (mm) and number of new branch candles (current growth), were measured for each test plant, on the day prior to the initial SAR application. These measurements were repeated at the end of the experiment. Foliar injury (chlorosis or necrosis) was assessed at this time. Net photosynthesis (umol $CO_2m^{-2}s^{-1}$) was determined using a Licor 6200 P.S. analyzer for a selected candle on each seedling at the end of the experiment (April 20 and again on April 22).

All current-year and one-year-old needles were removed from each seedling one week after terminating treatment applications. The foliage was then oven-dried for 48 hours at 80°C and total dry weight was recorded. Theses foliar samples were analyzed for the following elements: N, P, K, Ca, Mg, Al, Mn, Zn, Fe, and S using standard techniques. Whole plants were then harvested (shoot and root material)and oven-dried. Total dry shoot and root weights were determined . Shoot to root ratios were calculated from these weights.

For all data, anaysis of variance and regression analysis were performed using a computer statistical package (Statgraphics 2.1, STSC Plus*Ware, Maryland, U.S.A.).

RESULTS:

All test plants received a total of 210 mm of SAR, in 26 separate events, during the ten week experiment (Table 3). Selected seedlings were exposed to ten ozone fumigations during this period. No visible injury was observed on any of the test plants exposed to SAR alone, or in combination with ozone. The treatments had no significant effect on physical growth parameters measured; i.e. increases in height or stem diameter, dry biomass of current-year and one-year-old foliage, total shoot and root dry weight, and shoot to root ratio were not affected (Figures 1 to 7).

Mean foliar concentrations of the elements N, P, K, Ca, Mg, Mn, Al, and S accumulated in both current-year and one-year-old needles of test plants are shown in Figures 8 to 15 respectively. There were no significant treatment effects due to either SAR or ozone on foliar concentrations of the above elements in either current-year or one-year-old needles. SAR did not have a significant effect on foliar N concentrations (Figure 8). However, foliar concentrations of P, K and Mg were consistently higher in current year needles compared with one-year-old needles (Figure 9, 10, 12). In contrast, foliar concentrations of the elements, Ca and Al, were much greater in the one-year-old needles than in current-year needles (Figures 11 and 14). Like N, foliar concentrations of S and Mn did not differ significantly by treatment or by needle age (Figure 13 and 15).

Mean net P.S., which was determined one day after treatments were terminated (April 20), and again two days later (April 22), are shown in Figures 16 and 17 respectively. Although a trend is apparent in each of the two sets of measurements, the trends were not statistically significant. On both days, net photosynthesis (P.S.) appeared to be higher in seedlings exposed to SAR alone compared with plants which were also exposed to ozone. For seedlings exposed only to SAR, the pH 5.6 SAR-treated plants appeared to have a higher mean net P.S. than occurred in test plants subjected to either pH 4.3 or pH 3.2 SAR (refer to Figures 16 and 17). It should be noted that variability in net P.S. measurements amongst selected branch candles was high.

A regression was performed comparing net P.S. for selected branch candles (current-year growth) vs. photon flux density (umol $m^{-2}s^{-1}$) and is shown in Figure 18. Ambient light levels varied during the two periods when P.S. measurements were made; however, the regression slope is not significant (r= -.15). These data indicate that observed differences in net P.S. were not attibutable to variations in light conditions.

DISCUSSION:

The experimental results indicate that growth of white spruce seedling growth was not affected significantly by exposure to SAR, alone or in combination with ozone during the ten week greenhouse experiment. Variability in physical measurements between individual test plants within treatment groups was high. This can obscure subtle treatment effects. In a similar greenhouse study, white spruce seedlings, which were subjected to SAR (pH 2.6, 3.6, 4.6, 5.6, control) each week for 7 weeks, showed no statistically significant reduction in growth rate (Abouguendia and Baschak, 1987). Similarly, like the MOE study, no macroscopic, foliar injury symptoms were evident and nutrient levels remained unaffected (ibid). In another greenhouse experiment, Seiler and Pagnelli (1987), observed that red spruce seedling growth was unaffected by pH 3.0 SAR treatments vs. pH 4.5 SAR applied during a 19 week period. The investigators concluded that the duration of the experiment was not long enough to detect treatment effects.

In an early study by Davis and Wood (1972), white spruce seedlings were resistant to ozone fumigations as high as 250 ppb for periods of four to eight hours duration. The seedlings showed no chlorotic mottling or tip necrosis observed on other more sensitive conifer species, such as eastern white pine (Pinus strobus). Two-year-old, potted Sitka spruce (Picea sitchensis (Borg) Carr.) were exposed to controlled ozone fumigations (0.05, 0.70, 0.12, 0.17 ppm ozone for 7 hr day^{-1}) from June 1 to August 30, 1986 (Lucas et. al. 1988). The seedlings showed no apparent significant growth effects for shoot height or diameter during or after the ozone fumigations.

In a more recent study, Laurence et. al. (1989) exposed one-year-old red spruce seedlings to ozone at 0.5, 1, 1.5 and 2 times ambient levels (12 hr mean = 0.038 ppm) and SAR (pH 3.1, 4.1, 5.1) in open-topped chambers in the field during the months of July through September 1987. Neither pollutant caused significant effects on height, branch number and length, dry weight of needles, stems, or roots, and rate of photosynthesis.

No significant SAR or ozone treatment effects were observed in foliar concentrations of N, P, K, Ca, Mg, Al, Mn, or S. For all treatments, foliar concentrations of N, P, K an Mg were greater in current-year needles compared with one-year-old needles. Both P and K are highly mobile plant nutrients and are important in the production of new needle tissue. The element Mg, which is a crucial component of chlorophyll, was shown to be easily leached from Norway spruce foliage by acidic precipitation and ozone which can injure chloroplasts which then lose their ability to store Mg (Rothe et. al. 1988). However, foliar concentrations of Mg in both the current-year and one-year-old needles were above the generally accepted threshold concentration of 700 ug g^{-1} below which decline symptoms can occur (Zottl and Huttl, 1985).

Calcium, which is accumulated over time in cell walls and membranes, was present in higher concentrations in the one-year-old needles compared with current-year needles for all treatments. Although Al had accumulated in one-year-old needles, foliar Ca deficiencies were not observed in the white spruce seedlings.

Shortle and Smith (1988) showed that increases in Al concentration in the fine root environment, due to increased soil acidification will reduce Ca uptake in red spruce through competition for binding sites in the cortical apoplasts of fine roots. In a study conducted by Joslin et. al. (1988), Al tissue concentrations in red spruce and Norway spruce trees were analyzed from various sites in both North America and Europe. The authors determined that Al concentrations in roots were two orders of magnitude higher than in foliage and concluded that fine roots (<1.0 mm), which usually had the highest Al accumulations, appeared to be the best phyto-indicators of available Al in soil. The MOE study also provided circumstantial evidence that Al interferes with Ca and Mg uptake and transport (Joslin et. al. 1988).

Reductions of Ca, as well as Mg, occurred in red spruce seedling roots due to interference by aluminum in nutrient solution (Thornton et. al. 1987). Root elongation of Norway spruce was inhibited, and Ca and Mg root concentrations were reduced, by Al in roots of seedlings grown in nutrient solutions (Godbold et. al. 1988). In another study, Ca and Mg concentrations in roots of Norway spruce seedlings decreased with increasing Al concentration in nutrient solution. However, Ca and Mg concentrations were not lower in shoots except at the highest treatment level of Al (10 mM) (Asp et. al. 1988). Results of these studies demonstrate the importance of sampling fine roots, in addition to foliage, in order to better determine Al effects on seedling growth that may occur from increased soil acidification by SAR. Root analyses is warranted in the multi-year field study.

Mean net photosynthesis was not affected significantly by exposure to SAR, alone or in combination with ozone, in the current experiment, although mean net P.S. of ozone-treated seedlings was less than that of seedlings exposed to SAR only. In a similar study, Laurence et. al. (1989), was unable to detect significant ozone effects on photosynthetic rates of red spruce seedlings. The authors attributed this result to either the limited number of measurements made during the experiment or to the inherently low rate of gas exchange that is found in conifers compared to herbaceous plants. Reich and Amundson (1985) suggested that significant ozone effects can be observed on conifers only after the cumulative stress of exposure over several years reached a level comparable to that experienced by a rapidly photosynthesizing crop plant during one growing season.

Production of current-year needles is dependent on bud formation and utilization of photosynthates stored in roots during the previous growing season. This may at least partially explain why the application of SAR and ozone to the spruce seedlings during the spring of 1987 did not have a direct effect on growth and needle development at that time. Further research is warranted to determine if exposure to SAR and ozone treatments during the growing season will affect the production and storage of photosynthates for bud formation and spring flush in the following year. This factor will be investigated in the multi-year study which will utilize mobile rain exclusion canopies to expose potted white spruce seedlings to SAR and ozone in established field plots.

CONCLUSIONS

Results of the preliminary experiment did not show significant treatment effects on growth, nutrient status or net photosynthesis of the white spruce seedlings tested. This lack of significant treatment effects demonstrates that the ten week duration of the MOE greenhouse experiment was insufficient to establish conclusive treatment effects on growth. Subtle growth effects may be observed over the duration of a long term study. It is anticipated that significant treatment affects may be determined using rain exclusion canopies and blower systems to apply SAR and ozone to potted white spruce seedlings over several growing seasons. Also, in addition to foliage, white spruce seedling roots should be sampled in order to determine both Al toxicity to root growth and root uptake of Ca and Mg.

REFERENCES

Abouguendia, Z.M. and L.A. Baschak, 1987. Response of two western Canadian conifers to simulated acidic precipitation. Water, Air, Soil Pollut. 33: 15.

Asp, H., B. Bengtsson, P. Jensen, 1988. Growth and cation uptake in spruce (Picea abies Karst.) grown in sand culture with various aluminum contents. Plant and Soil 111: 127.

Chan, W.H., A.J.S. Tang, M.A. Lusis, 1983. Precipitation and wet deposition fields of pollutants in Ontario, Sept. 1980 to Dec. 1981. APIOS Report ARB-61-83-ARSP, Ont. Min. of Environment, Toronto, Ontario.

Davis, D. and F.A. Wood, 1972. The relative susceptibility of eighteen coniferous species to ozone. Phytopath. 62: 14.

Godbold, D.L., K. Dictus, A. Hutterman, 1988. Influence of aluminum and nitrate on root growth and mineral nutrition of Norway spruce (Picea abies) seedlings. Can.J. For. Res. 18: 1167.

Hornbeck, J.W. and R.B. Smith, 1985. Documentation of red spruce growth decline. Can. J. For. Res. 15: 1199.

Hutchinson, T.C., L. Bozic, G. Munoz-Vega, 1986. Response of five species of conifer seedlings to aluminum stress. Water, Air, Soil Pollut. 31: 283.

Jagels, R., 1986. Acid fog, ozone and low level spruce decline. IAWA Bull. 7: 299.

Johnson, A.H., 1983. Red spruce decline in northeastern U.S.A.: hypothesis regarding role of acid rain. J.A.P.C.A. 33: 1049.

Joslin, J.D., J.M. Kelly, M.H. Wolfe, L.E. Rustad, 1988. Elemental patterns in roots and foliage of mature spruce across a gradient of soil aluminum. Water, Air, Soil Pollut. 40: 375.

Krause, G.H.M., 1988. Ozone-induced nitrate formation in needles and leaves of Picea abies, Fagus sylvatica and Quercus robur. Envir. Pollut. 52: 117.

Laurence, J.A., R.J. Kohut, R.G. Amundson, 1989. Response of red spruce seedlings exposed to ozone and simulated acidic precipitation in the field. Arch. Envir. Contam. Toxicol. 18: 285.

Linzon, S.N., 1985. Forest damage and acidic precipitation. In: E.B. Eddy Distinguished Lecture Series, Nov. 1985, Fac. of Forestry, Univ. of Toronto.

Lucas, P.W., D.A. Cottam, L.J. Sheppard, B.J. Francis, 1988. Growth responses and delayed winter hardening in Sitka spruce following summer exposure to ozone. New Phytol. 108: 495.

Nosko, P., P. Brassard, J.R. Kramer, K.A. Hershaw, 1988. The effect of aluminum on seed germination and early seedling establishment, growth and respiration of white spruce (Picea glauca). Can. J. Bot. 66: 2305.

Percy, K., 1986. The effects of simulated acid rain on germination capacity, growth and morphology of forest tree seedlings. New Phytol. 104: 473.

Reich, P.B. and R.G. Amundson, 1985. Ambient levels of ozone reduce net photosynthesis in tree and crop species: Inplications for future work. Science 230: 566.

Roth, G.M., H. Weil, M. Geider, P. Pfennig, V. Wilhelmi, W.D. Maurer, 1988. Nutrient element and carbohydrate status of Norway spruce at Mt. Kleiner Feldberg in Taunus exposed to air pollution and soil acidification. Eur. J. For. Path. 18: 98.

Seiler, J.R. and D.J. Paganelli, 1987. Photosynthesis and growth response of red spruce and loblolly pine to soil-applied lead and simulated acid rain. For. Sci. 33: 668.

Siccama, T.G., M. Bliss and H.W. Vogelmann, 1982. Decline of red spruce in the Green Mountains of Vermont. Bull. Torrey Bot. Club 109: 162.

Thornton, F.C., M. Schaedle, D.J. Raynal, 1987. Effects of aluminum on red spruce seedlings in solution culture. Envir. and Exp. Bot. 27: 489.

Ulrich, B., R. Mayer, P.K. Khanna, 1980. Chemical changes due to acid precipitation in a loess-derived soil in central Europe. Soil Sci. 130: 193.

Ulrich, B., 1981. Eine okosystem hypothese uberdie Ursache des Tannersterbens (Abies alba Mill.) Forstiviss Cbl. 100: 228.

Ulrich, B., 1983. Soil acidity and its relations to acid deposition. In: B. Ulrich and J. Pankrath (eds.) 1983. Effects of air pollution on forest ecosystems. Reidel Publ. Dordrecht pp. 1-32.

Van Breemen, N., 1985. Acidification and decline of central European forests. Nature 315: 6.

Vogelmann, A.F. and B.N. Rock, 1988. Anatomy of red spruce needles from forest decline sites in Vermont. Envir. and Exp. Bot. 28: 19.

Weinstein, L.H., R.J. Kohut, J.S. Jacobson, 1987. Research at Boyce Thompson Institute of the effects of ozone and acidic precipitation on red spruce. In: Proceedings of 80th annual meeting of APCA, New York, N.Y., June 21-26, 1987. 87-34.1.

Zottl, H.W. and R.F. Huettl, 1985. Schadsymptome und Ernahrungszustand von Fichtenbestanden im suddeutschen Alpenvorland. Allg. Forstz. 40: 197.

Zottl, H.W. and R.F. Huettl, 1986. Nutrient supply and forest decline in southwest Germany. Water, Air, Soil Pollut. 31: 449.

Table 1. Chemistry of soil[*] utilized in SAR/ozone experiment with potted white spruce seedlings.

Soil Parameter	Concentrations[+]
pH (water extract)	5.1
pH (CaCl extract)	4.5
Ca (mg/g)	2.2
Mg (mg/g)	2.3
K (mg/g)	0.4
Al (mg/g)	14.3
% organic C	2.4
% Sand	37
Silt	58
Clay	5

[*] B horizon soil collected from Plastic Lake site near Dorset.

[+] Total concentrations (dry weight).

Table 2. Chemical composition of simulated acidic rain (SAR) solution.

SAR*	H^+ ueq L^{-1}	pH	Acid vol. ml L^{-1} H_2SO_4	HNO_3
1	12	5.6	0	0
2	50	4.3	1.001E-3	8.849E-4
3	650	3.2	1.301E-2	1.151E-2

* All simulated rain solutions contained the following background ions (mg $L^{-1)}$

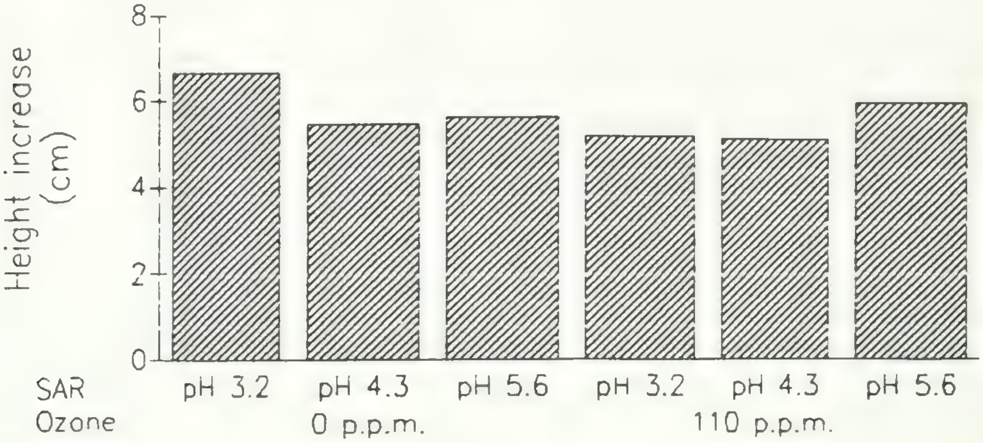
K - 0.063 mg L^{-1}	Ca - 0.501 mg L^{-1}
Mg - 0.103 mg L^{-1}	Na - 0.100 mg L^{-1}
NH^4 - 0.744 mg L^{-1}	Cl - 0.023 mg L^{-1}
SO^4 - 2.771 mg L^{-1}	NO^3 - 1.385 mg L^{-1}

S/N ratio = 2:1

Table 3: Treatment Application schedule

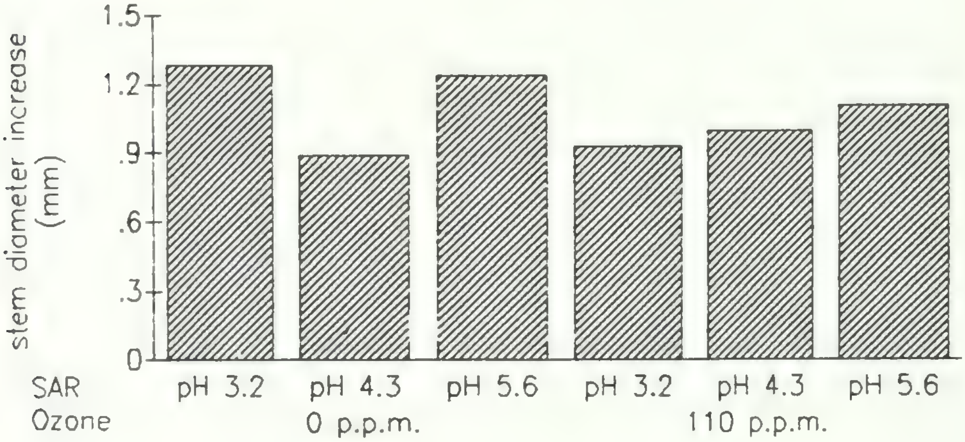
	SAR (mm)	Ozone fumigation	Activities
Oct. 14, 1987			transplanted to
			Dorset soil
Jan. 18, 1988			moved the pots
			to the greenhouse
Feb. 9, 1988			physical measurements
Feb. 10	7		
Feb. 11		110 ppb peak	
Feb. 12	9		
Feb. 15	8		
Feb. 16		110 ppb peak	
Feb. 17	8		
Feb. 18	8		
Feb. 22	8		
Feb. 23		110 ppb peak	
Feb. 24	8		
Feb. 26	8		
Feb. 29	8		
Mar. 1		110 ppb peak	
Mar. 2	8		
Mar. 4	8		
Mar. 7	8		
Mar. 8		110 ppb peak	
Mar. 9	8		
Mar. 11	8		
Mar. 14	8		
Mar. 15		110 ppb peak	
Mar. 16	8		
Mar. 18	8		
Mar. 21	8		
Mar. 22		110 ppb peak	
Mar. 23	8		
Mar. 25	8		
Mar. 28	8		
Mar. 29		110 ppb peak	
April 2	8		
April 4	8		
April 5		110 ppb peak	
April 6	8		
April 15	8		
April 18	8		
April 19		110 ppb peak	
April 20			LICOR measurements
April 21			physical measurements
April 22			LICOR measurements

Figure 1: Height increase of potted white spruce seedlings exposed to SAR alone or in combination with ozone. Feb. 10 – April 28, 1988.



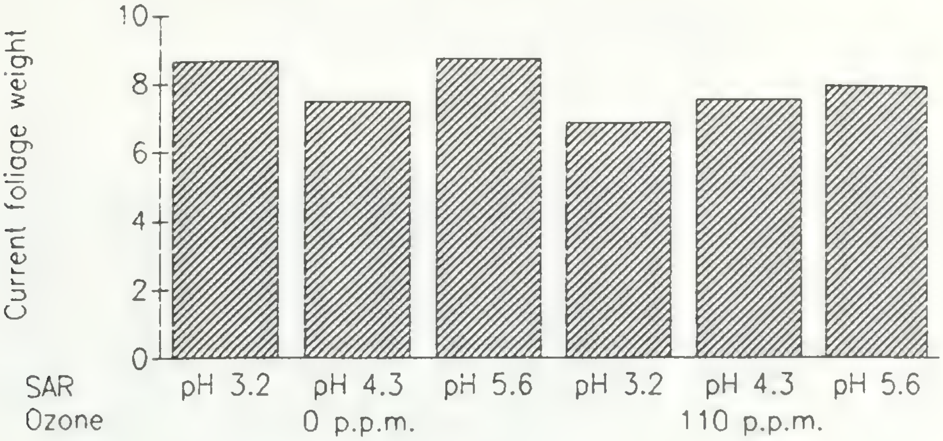
columns are not significantly different (p< .05)

Figure 2: Increase in stem diameter of potted white spruce seedlings exposed to SAR alone and in combination with ozone. Feb. 10 – April 28, 1988.



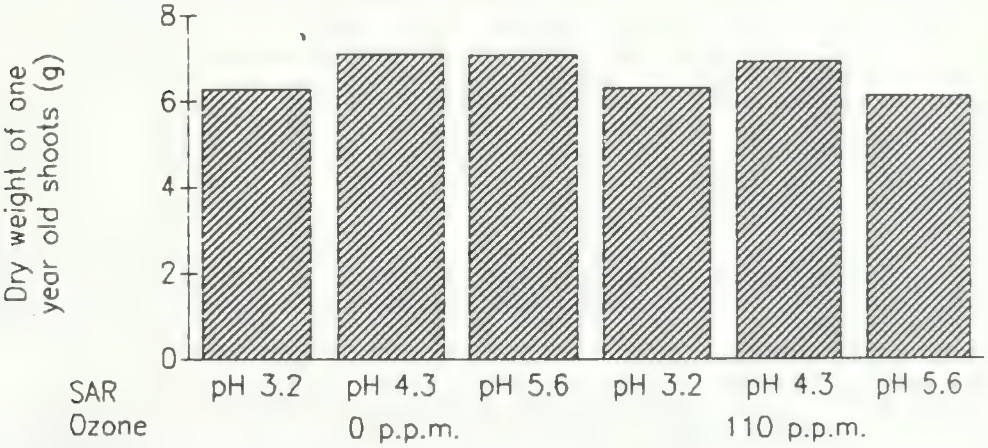
columns are not significantly different (p< .05)

Figure 3: Weight of current foliage of potted white spruce seedlings exposed to SAR alone or in combination with ozone. Feb. 10 – April 28, 1988.



columns are not significantly different (p< .05)

Figure 4: Dry weight of one year old foliage of potted white spruce foliage exposed to SAR alone or in combination with ozone. Feb. 10 – April 28, 1988.



columns are not significantly different (p< .05)

Figure 5: Dry weight of shoots of potted white spruce seedlings exposed to SAR alone or in combination with ozone. Feb. 10 – April 28, 1988.

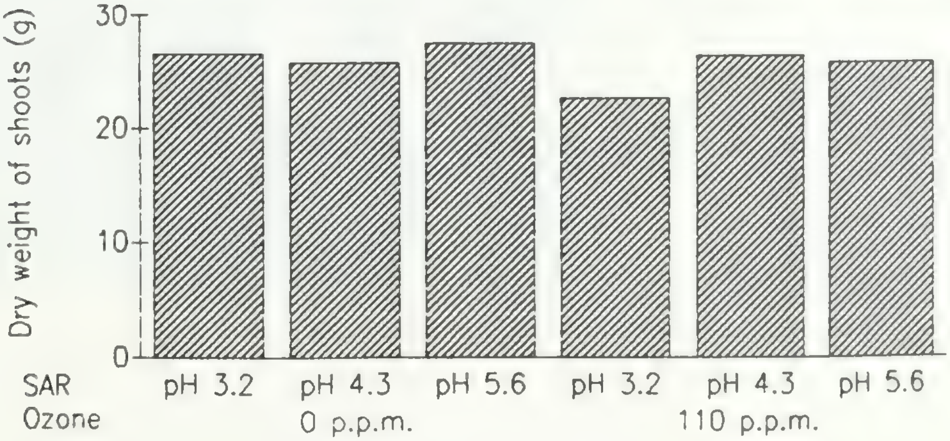
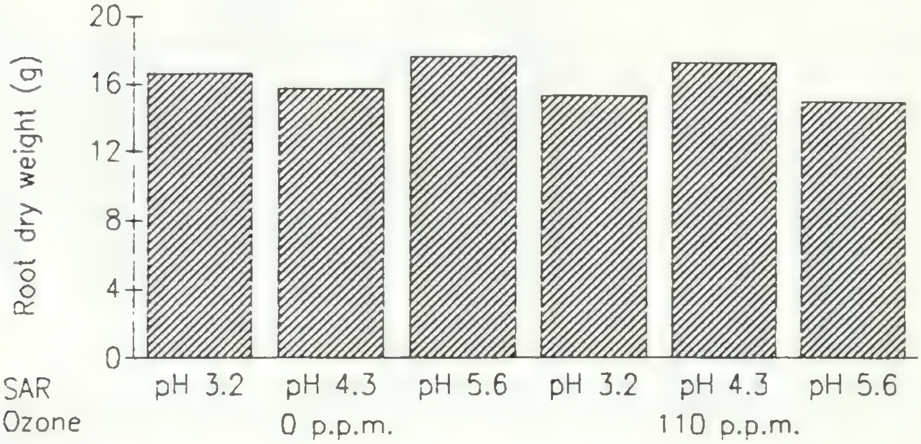
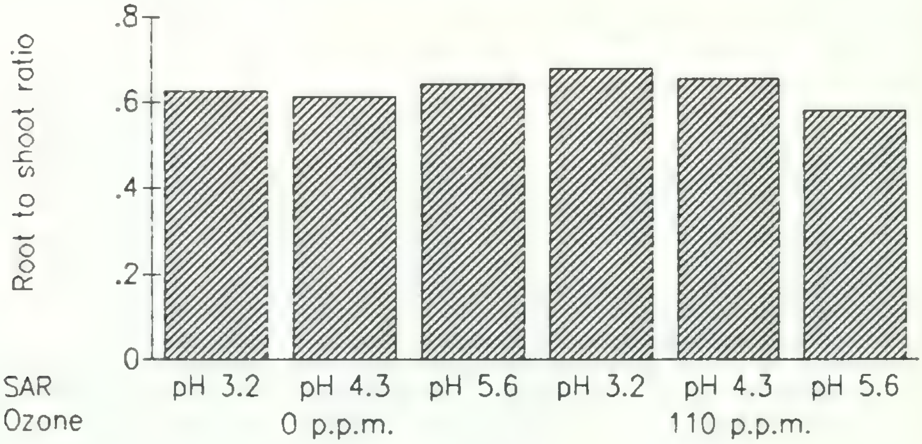


Figure 6: Root dry weight of potted white spruce seedlings exposed to SAR alone or in combination with ozone. Feb. 10 – April 28, 1988.



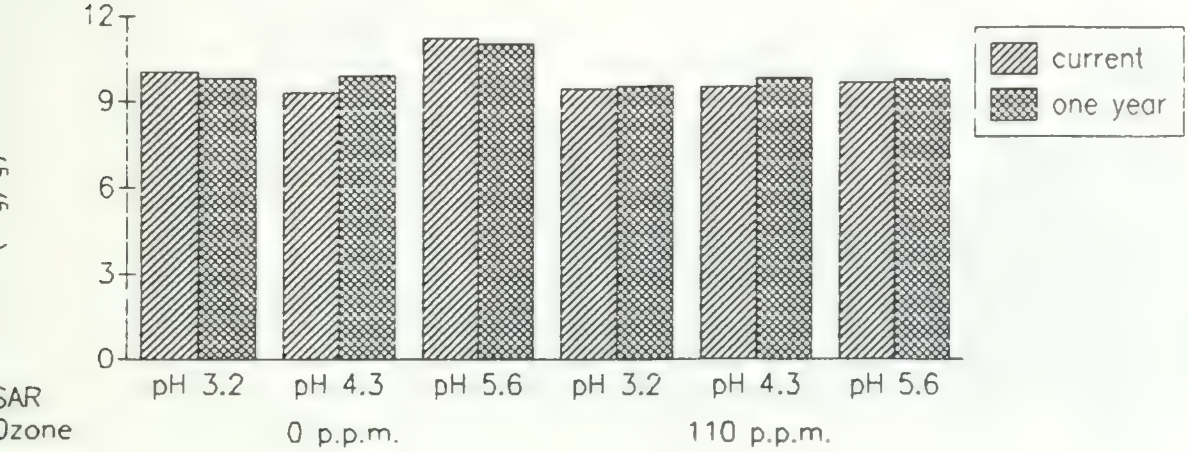
columns are not significantly different (p< .05)

Figure 7: Root to shoot ratio of potted white spruce seedlings exposed to SAR alone or in combination with ozone. Feb. 10 – April 28, 1988.

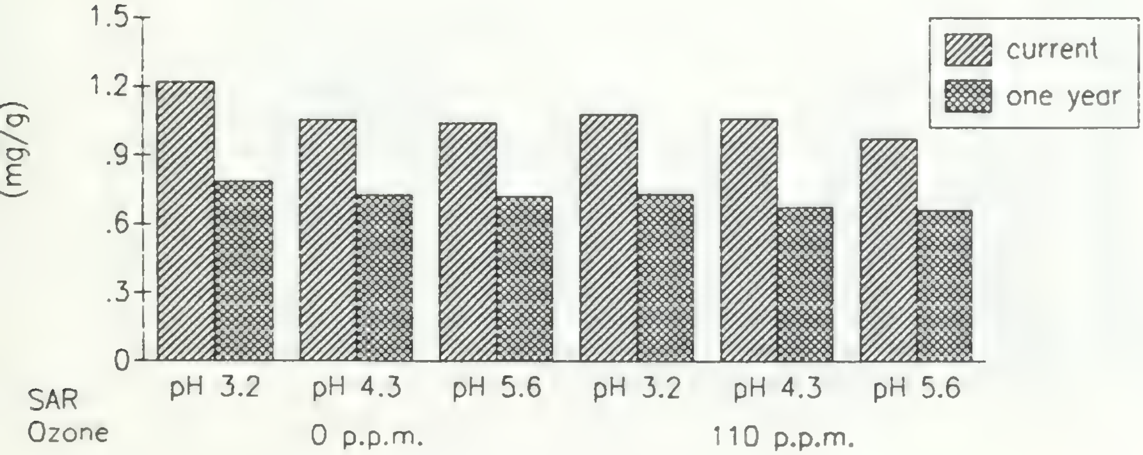


columns are not significantly different (p< .05)

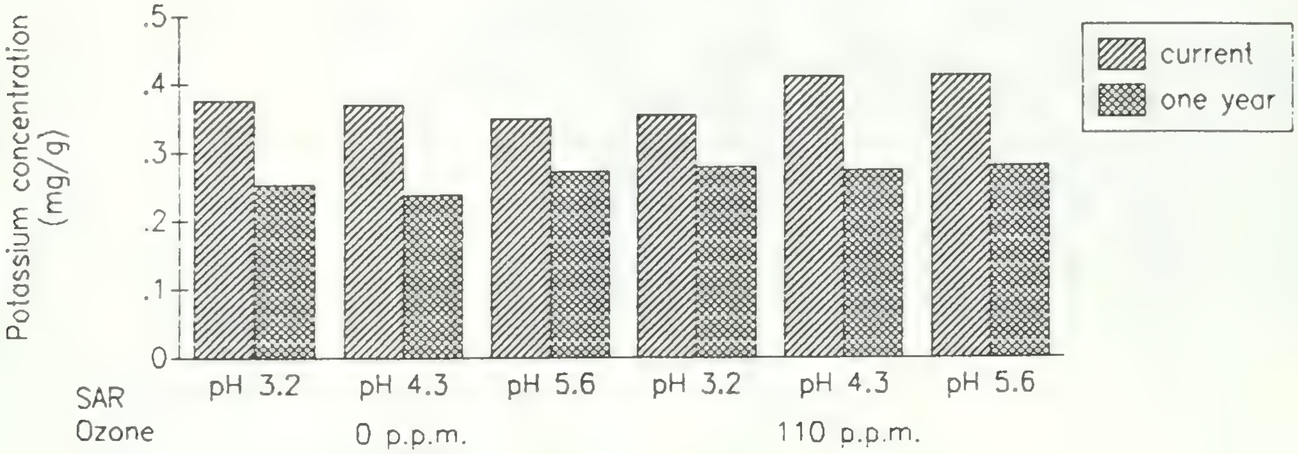
Figure 8: Foliar nitrogen concentration (mg/g) in potted white spruce seedlings exposed to SAR alone or in combination with ozone. (current and one year needles) Feb. 10 to April 20, 1988.



gure 9: Foliar phosphorus concentration (mg/g) in potted white spruce seedlings d to SAR alone or in combination with ozone. (current year and one year old needles) Feb. 10 to April 28, 1988.

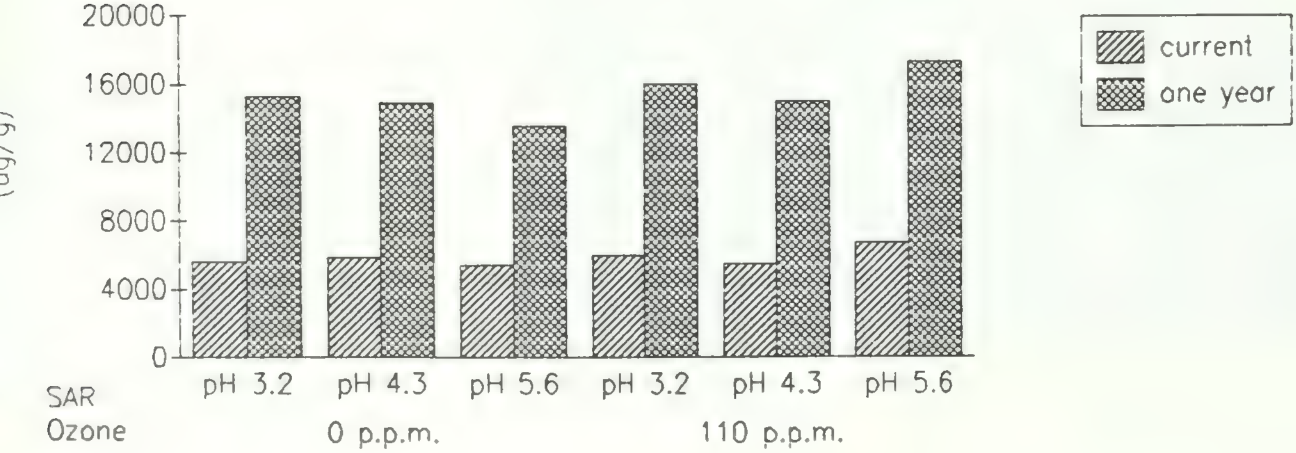


gure 10: Foliar potassium concentration (mg/g) in potted white spruce seedlings
posed to SAR alone or in combination with ozone. (current and one year needles)
Feb. 10 to April 28, 1988.



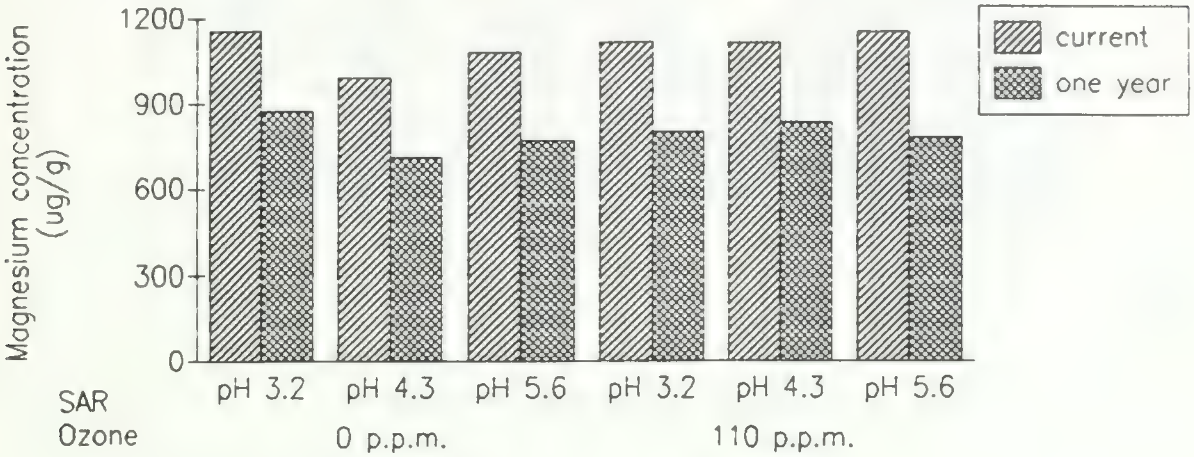
collumns within a foliar age catagory, are not significantly different (p<.05)

Figure 11: Foliar calcium concentration (ug/g) in potted white spruce seedlings
exposed to SAR alone or in combination with ozone. (current and one year needles)
Feb. 10 to April 28, 1988.



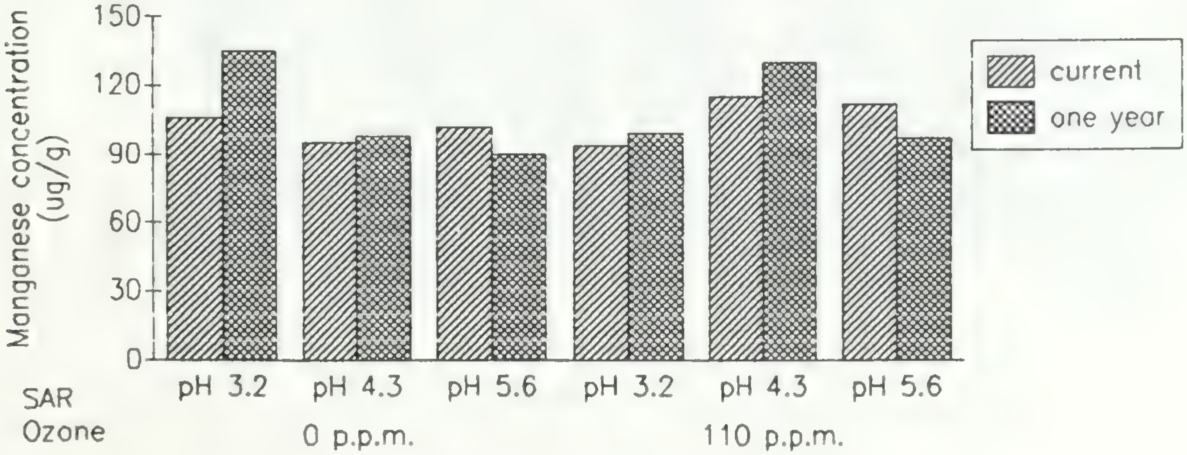
collumns within a foliar age catagory, are not significantly different (p<.05)

gure 12: Foliar magnesium concentration (ug/g) in potted white spruce seedlings
xposed to SAR alone or in combination with ozone. (current and one year needles)
Feb. 10 to April 28, 1988)



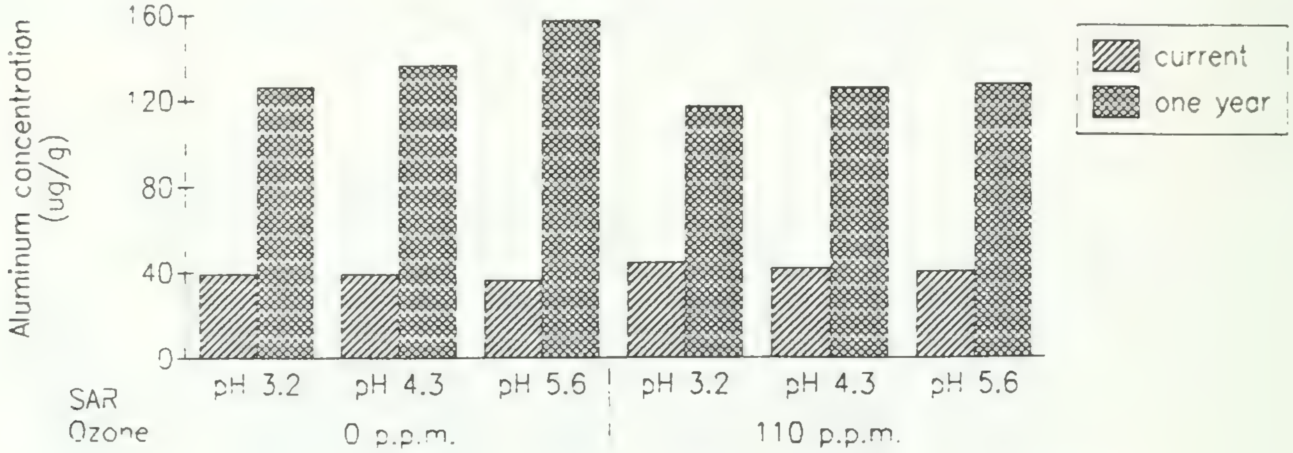
collumns within a foliar age catagory, are not significantly different (p<.05)

ure 13: Foliar manganese concentration (ug/g) in potted white spruce seedlings
exposed to SAR or in combination with ozone. (current and one year needles)
Feb. 10 to April 28, 1988.



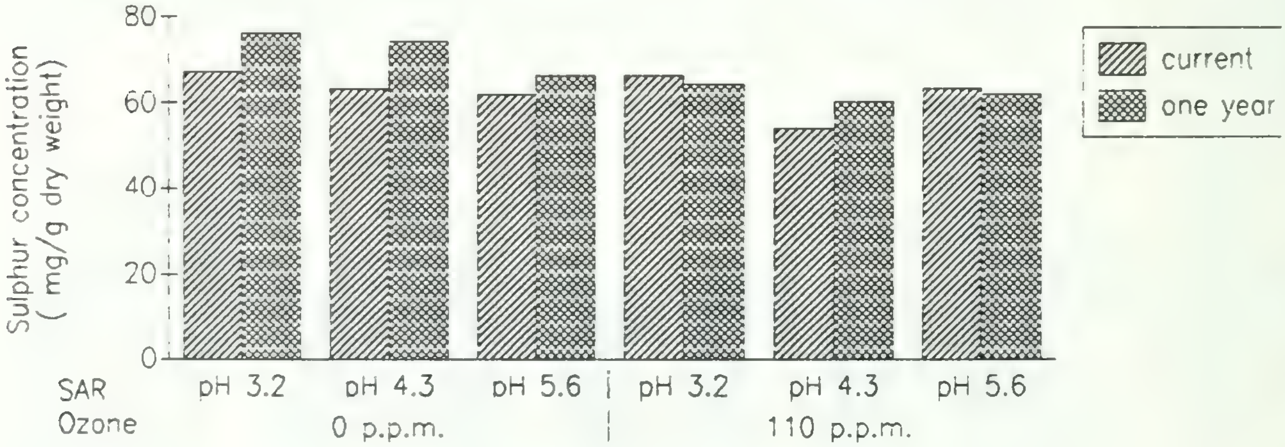
collumns within a foliar age catagory, are not significantly different (p<.05)

Figure 14: Foliar aluminum concentration (ug/g) in potted white spruce seedlings exposed to SAR alone or in combination with ozone. (current and one year needles) Feb. 10 to April 28, 1988.



collumns within a foliar age catagory, are not significantly different (p<.05)

Figure 15: Foliar sulphur concentration (mg/g dry weight) in potted white spruce seedlings exposed to SAR alone or in combination with ozone. (current and one year needles) Feb. 10 to April 28, 1988.



collumns within a foliar age catagory, are not significantly different (p<.05)

Figure 16: Net photosynthesis of white spruce seedlings, measured on April 20, 1988, which had been exposed to SAR alone or in combination with ozone

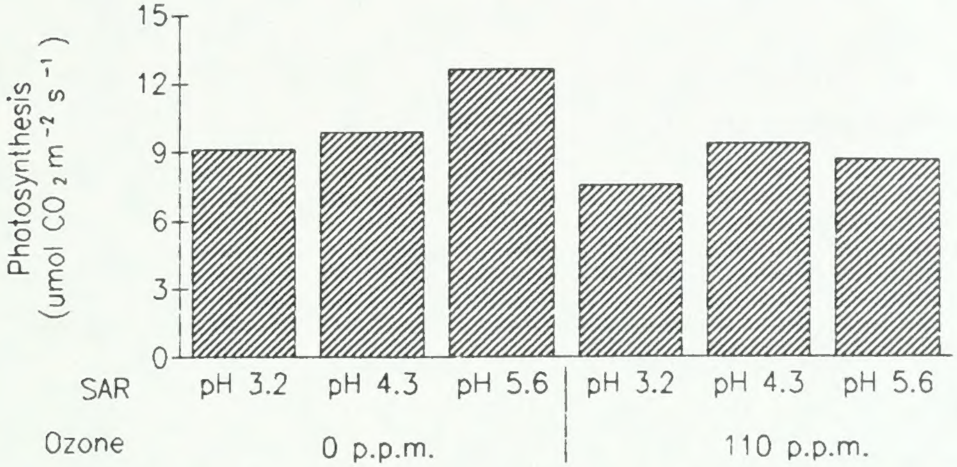


Figure 17: Net photosynthesis of white spruce seedlings, measured on April 22, 1988, which had been exposed to SAR alone or in combination with ozone

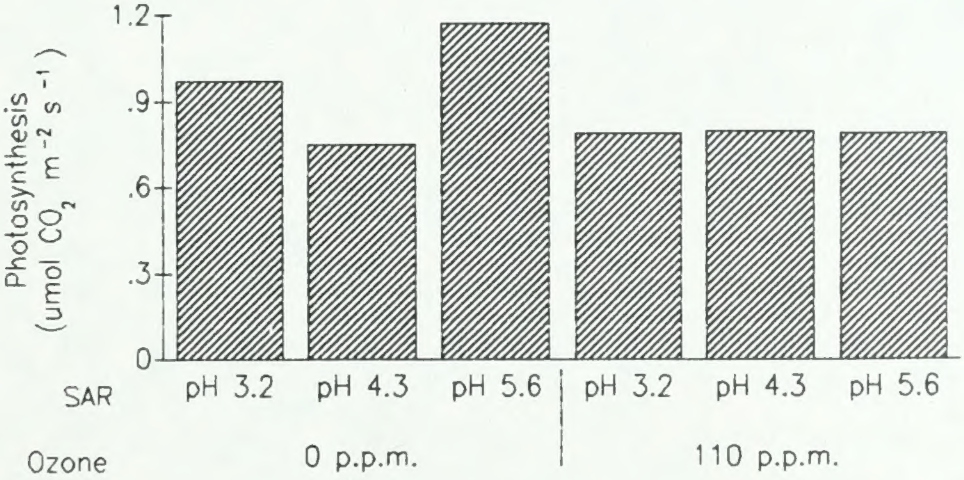


Figure 18: Regression comparing photosynthesis to photon flux density for white spruce seedlings growing in the greenhouse; April 1988.